LC-MS/MS rapid quantification and screening method for 30 mycotoxins in animal feed

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System

Zhai Nannan, Jia Yanbo, Jin Wenhai
SCIEX, China

In recent years, animal mycotoxin poisoning incidents have occurred frequently in China and around the world[1], causing huge economic losses to the agriculture industry. Feed mycotoxin contamination is an ongoing global problem that seriously affects animal health and production and poses major food safety hazards. Controlling the mycotoxin contamination of animal feed is a common goal in the industry.

On October 14, 2017, China's General Administration of Quality Supervision, Inspection and Quarantine and the National Standardization Management Committee issued the "Feed Hygiene Standards"[2] (GB13078-2017), which stipulates seven mycotoxin limit requirements in animal feed. This added to the list of global regulations of mycotoxins in feed that already exists in the European Union and the United States. This is significant, as China's role in the global food supply is ever-growing. To not only meet regulatory guidelines, but to also maintain consumer protection and promote safe practices during the growth of agriculture and animal husbandry industries, it is important to have methods that monitor for known mycotoxins outside of the regulatory guidelines.

This work focuses on the detection of mycotoxins in animal feed. On the SCIEX Triple Quad 3500 System, a rapid determination method of 30 mycotoxins was established. This method provides a simple and quick solution for the detection of mycotoxins in the feed.

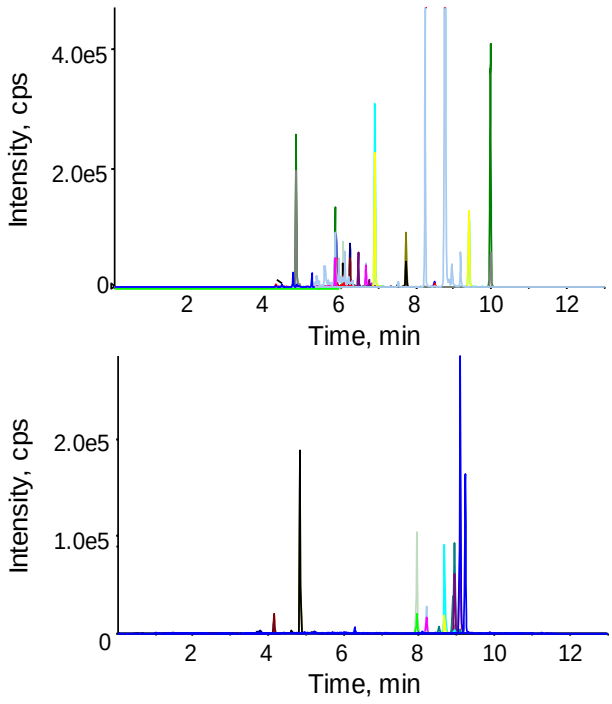

Figure 1. Typical chromatograms of 30 mycotoxins in pig feed. (Top) Extracted ion chromatograms (XICs) from the positive ionization mode. (Bottom) XICs from negative mode experiment.

Key features of the mycotoxin workflow

- Efficient sample preparation for animal feed, providing high extraction recovery rates
- Easy to implement
- Fast polarity switching: the SCIEX Triple Quad 3500 System enables the use of a single injection workflow, with both positive and negative mode acquisition to cover all 30 analytes
- Fast run time of 13 minutes, providing high sample throughput
- Acquisition method details provided, including chromatographic and mass spectrometry conditions for 30 mycotoxins
- Method sensitivity that fully meets the requirements of global mycotoxin regulations for animal feed

Methods

Sample preparation: Samples were prepared using solid phase extraction (SPE) with a Cleanert MC (400 mg/2 mL) or a Cleanert SAX SPE Column (500 mg/6 mL) from Agela Technologies.

Sample extraction:

1. Weigh 2.5 g of homogenized sample into a 50 mL centrifuge tube.
2. Add 10 mL of acetonitrile/water/formic acid solution (85:15:0.1).
3. Extract by ultrasonication, then vortex to homogenize.
4. Centrifuge the extracted sample and remove the supernatant for further cleanup.

Sample cleanup:

Method 1 — for fumonisin B1, B2, B3 and ochratoxin:

1. To a 2 mL aliquot of the extracted supernatant solution, add ammonia to adjust to pH 6–8.
2. Activate the Cleanert SAX column with 5 mL methanol, and then equilibrate with 5 mL water.
3. Load the extracted supernatant and then rinse the column with 8 mL methanol/water (60:40), followed by 3 mL methanol, and then elute with methanol containing 1% acetic acid.
4. Blow down the sample to dryness with nitrogen and reconstitute with 125 µL acetonitrile/water = 5/95 (v/v).

Method 2 — for the other 26 toxins

1. Pass the remaining 1 mL aliquot of the original sample extract through a Cleanert MC column
2. Blow down the purified sample to dryness with nitrogen and reconstitute with 125 µL acetonitrile/water = 5/95 (v/v).
3. Combine the reconstituted sample with that from Method 1 for LC-MS/MS analysis

Chromatography: Sample separation was performed using an ExionLC™ System and a Phenomenex Kinetex C18 column (100 × 3.0 mm, 2.6 µm). The flow rate was 0.5 mL/min, the column temperature was held at 40 °C and the injection volume was 20 µL. The gradient elution program is listed in Table 1.

Mass spectrometry: MS analysis was performed on the SCIEX Triple Quad 3500 System equipped with the Turbo V™ Ion Source. Acquisition parameters are detailed in Table 2.

Table 1: LC gradient details.

Time (min)	A (%)	B (%)
0.0	*97*	*3*
1.0	*97*	*3*
2.0	*90*	*10*
4.0	*50*	*50*
9.0	*20*	*80*
9.1	*0*	*100*
11.0	*0*	*100*
11.1	*97*	*3*
13.0	*97*	*3*

Mobile phase A: water with 0.1% formic acid
Mobile phase B: methanol with 0.1% formic acid

Table 2. Mass spectrometer settings.

Parameter	Setting	Parameter	Setting
Acquisition mode	*MRM*	*GS1*	*50 psi*
Ionization mode	*ESI*	*GS2*	*65 psi*
Polarity	*Positive and Negative*	*CAD gas*	*Medium*
Ion Source Voltage	*5500 V -4500 V*	*Source Temperature*	*550°C*
CUR Gas	*30 psi*		

Results

Using standards, the MRM transitions and MS parameters for the 30 mycotoxins were optimized. Then the chromatographic conditions for the analysis were optimized to provide fast run times and good separation (Figure 1). Polarity switching was used to enable sensitive detection of all 30 compounds in a single injection, with a total run time of 13 minutes.

Two different sample preparation strategies were explored to ensure the highest efficiency and recovery for all 30 mycotoxins. Best results were obtained using a combination of two different SPE columns, as described in the methods section.

After optimization, recovery experiments were performed to characterize the final sample preparation strategy, where the extraction recovery rate of all compounds was found to be within 70–120% (Figure 2).

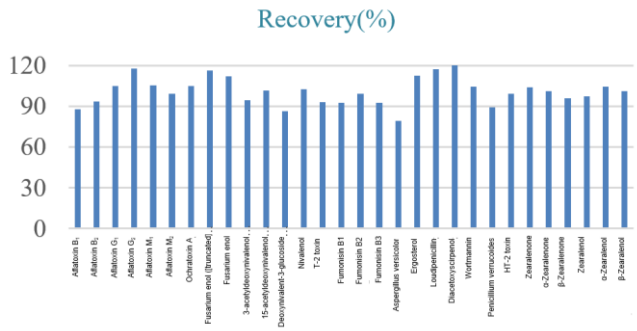

Figure 2: Extraction recovery for each of the mycotoxins analyzed. All calculated recoveries fell within 70–120% .

Calibration curves were generated to determine the lower limits of quantification (LLOQs) for each of the 30 mycotoxins. Very good linearity and reproducibility were obtained across the dataset. Example data for Fumonisin B1 is shown in Figure 3. Good linearity was observed across the interrogated concentration range of 1–40 µg/kg, with %CV across replicates <5%.

LLOQs for each of the compounds are outlined in Table 3, along with regulatory requirements from China, the EU and the US. The sensitivity of this complete method fully meets all outlined requirements for the detection sensitivity of mycotoxins in animal feed, and can monitor a suite of mycotoxins that are not yet regulated.

Table 3: List of method LLOQs and regulatory guidelines.

Compound	Method LLOQ (µg/kg)	Regulation China[2] (µg/kg)	Regulation EU[4] (µg/kg)	Guidance USA[5-7] (µg/kg)
Aflatoxin B1	*1*	*2*	*5*	*Total = 5*
Aflatoxin B2	*1*			
Aflatoxin G1	*0.5*			
Aflatoxin G2	*0.5*			
Aflatoxin M1	*0.5*			
Aflatoxin M2	*0.5*			
Aspergillus versicolor	*0.5*			
T-2 toxin	*0.1*	*2*		
Ergosterol	*1*			
Loudi penicillin	*0.1*			
Diacetoxyscirpenol	*10*			
Fusarium enol	*20*			
Deoxynivalenol	*20*	*100*	*900*	*1000*
3-acetyldeoxynivalenol	*10*			
15-acetyldeoxynivalenol	*10*			
Deoxynivalenol-3-glucoside	*20*			
Zearalenone	*5*		*100*	*NOG#*
α-zearalenone	*5*			
β-zearalenone	*2*			
Zearalenol	*2*			
α-zearalenol	*2*			
β-zearalenol	*2*			
Ochratoxin A	*1*	*5*	*50*	*NOG#*
Fumonisin B1	*1*	*B1+B2 = 50*	*B1+B2 = 5*	*B1+B2+B3 = 5000*
Fumonisin B2	*1*			
Fumonisin B3	*1*			
Wortmannin	*10*			
HT-2 toxin	*20*			
Nivalenol	*20*			
Penicillium verrucosum	*5*			

**- Lowest MRL across all feed types, #-FDA has no official guidance (NOG). Any sample positives for these mycotoxins are referred to the Center for Veterinary Medicine to determine if regulatory action is required*

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Fumonisin B1 1	1.0000	3 of 3	1.0142	0.0397	3.92	101.42
2	Fumonisin B1 1	2.0000	3 of 3	1.8915	0.0266	1.40	94.58
3	Fumonisin B1 1	5.0000	3 of 3	5.1004	0.2222	4.36	102.01
4	Fumonisin B1 1	10.0000	3 of 3	11.3009	0.4347	3.85	113.01
5	Fumonisin B1 1	20.0000	3 of 3	18.9296	0.3081	1.63	94.65
6	Fumonisin B1 1	40.0000	3 of 3	37.7365	0.8932	2.37	94.34

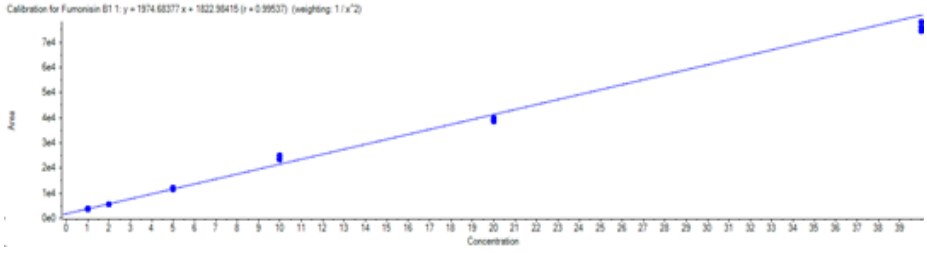

Figure 3: Reproducibility and linear results of Fumonisin B1. Calibration curves were generated for all mycotoxins across the concentration range of 1 – 40 µg/kg. Data shown above for Fumonisin B1. Statistics table for calibration curves showing high accuracy and %CV< 5%

Summary

A complete method was developed for the detection of mycotoxins in animal feed using the SCIEX Triple Quad 3500 System. Sample preparation was optimized for good recoveries of the 30 analytes from matrix, with 70–120% recovery. Single injection workflow with polarity switching enabled detection of all 30 mycotoxins within 13 minutes with high efficiency and high throughput. The method meets regulatory standards, while adding the ability to quantify mycotoxins that currently are not part of animal feed regulations for additional safety and security for consumers.

References

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5. Guidance for Industry and FDA: Advisory Levels for Deoxynivalenol (DON) in Finished Wheat Products for Human Consumption and Grains and Grain By-Products used for Animal Feed.
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7. Action Levels for Aflatoxins in Animal Feeds CPG Sec. 683.100.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

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