Simultaneous Analysis of 26 Mycotoxins in Grain on a SCIEX Triple Quad™ 3500 MS System

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Mycotoxins are secondary metabolites produced by a wide range of fungi known to contaminate a variety of food and agricultural commodities worldwide and have been recognized as a potential health threat to humans and animals. Many countries have regulations in place for mycotoxin detection and identification and their permissible limits. In China, the limits of mycotoxins in certain products are regulated by GB 2761 and in EU, mycotoxin limits are harmonized in the regulation for contaminants in foodstuffs EC 1881/2006 and the amended regulation EC 1126/2007. Regulations on food and environmental analysis require the analysis of contaminants using confirmatory techniques. Thus, there is a demand for powerful and rapid analytical methods that can detect very low concentrations of mycotoxins in a variety of sample matrices. In recent years, LC-MS/MS has gained popularity, becoming the method of choice, leveraging its ability to analyze a wider range of compounds in a single analysis coupled together with the high selectivity and sensitivity of Multiple Reaction Monitoring (MRM).

Traditionally, different classes of mycotoxins required different sample preparation techniques, making the process laborious and time consuming. Presented here is a single workflow to analyze 26 compounds simultaneously. This workflow consists of a simplified extraction procedure that does away with additional clean-up steps by immunoaffinity columns and couples it to high resolution LC separation and high sensitivity MS detection.

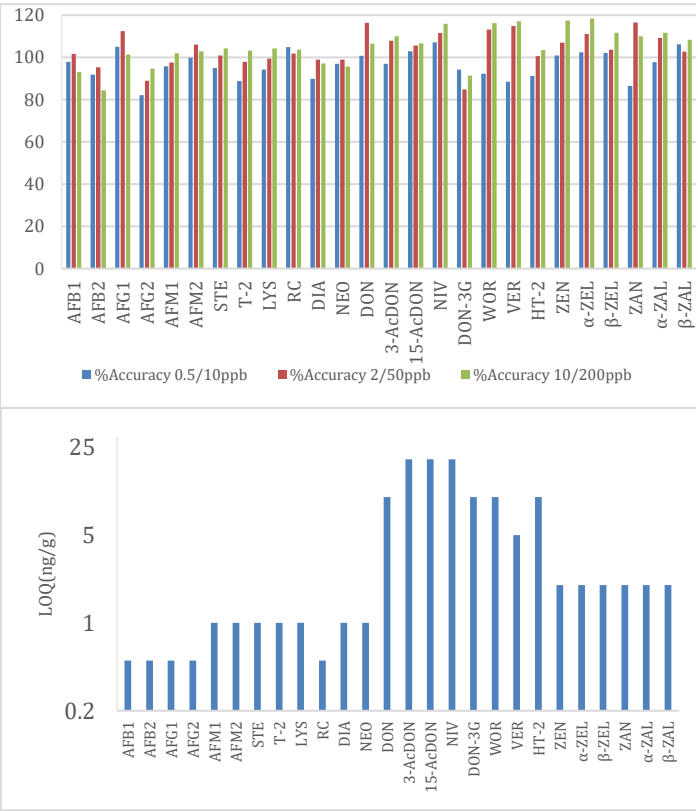

Figure 1. Accuracy and LOQ Values Shown for the Panel of Mycotoxins. Limits of Quantitation (LOQ) of all mycotoxins were found between 0.5 ng/g and 20ng/g. Accuracy assessed over three concentrations ranged from 80% to 120%. These measurements of performance demonstrate excellent sensitivity and accuracy for this assay.

Key Assay Attributes

- A fully integrated LC-MS/MS solution is presented to analyze 26 common mycotoxin residues simultaneously in relevant grain samples. Polarity switching ensures best coverage of relevant analytes.
- Simplified extraction procedure is described which does away with additional clean-up steps, saving time and labor at the front end of analysis.
- The method was validated for performance including sensitivity and robustness in different grain matrices.
- Limits of Quantitation (LOQ) of all mycotoxins were found between 0.5µg/kg and 20µg/kg. All LOQ meet the requirements of the grain Industry standard.

Experimental

Sample Preparation: Sample preparation was carried out in accordance to the vMethod SOP (P/N 5060674). Grain samples (corn, rice, wheat etc.) were first homogenized and 2.5g of sample was extracted using a mixture of acetonitrile and water. Once sonicated and centrifuged, the supernatant was passed through a Cleanert® MC SPE Cartridge (Agela Technologies, P/N ZS-MYT10-B) which contains a sorbent chemistry specially optimized for mycotoxins. The filtrate was then dried down and reconstituted for LC-MS analysis.

LC Conditions: Liquid chromatography analysis was performed using a SCIEX ExionLC™ AD UHPLC system. 20µL was injected onto a Phenomenex Kinetex C_{18} column (100mm X 2.1 mm, 1.7µm, P/N 00D-4475-AN). Mobile phase A contained water with 0.1% formic acid and mobile phase B contained methanol with 0.1%.

Table 1. LC Gradient Time Program. Flow rate at all steps was 0.3 mL/min, and the total run time was 13 minutes including re-equilibration.

Time (min)	%B
1.0	*3*
2.0	*10*
4.0	*50*
9.0	*80*
9.1	*99*
11.0	*99*
11.1	*3*
13.0	*3*

MS/MS Conditions: Electrospray ionization was carried out on SCIEX Triple Quad™ 3500 system with fast polarity switching. The Turbo V™ source was kept at a temperature of 550°C and the *Scheduled* MRM™ algorithm was used to analyze grain samples for 26 mycotoxins in a single injection by multiplexing the detection of multiple MRM transitions for signature fragments.

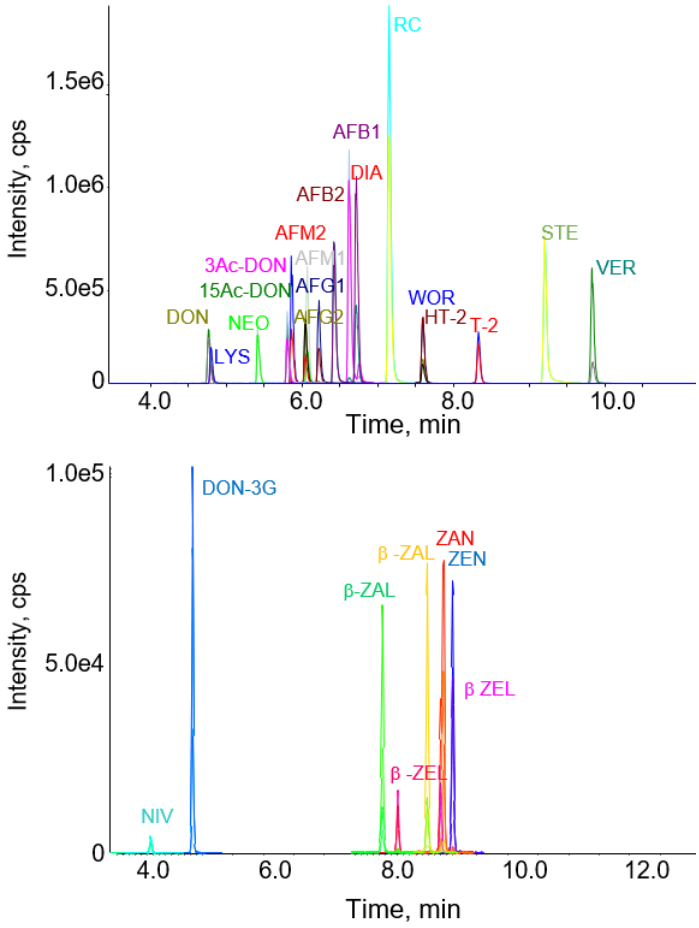

Figure 2. Chromatographic Profile for 26 Mycotoxins. Both positive and negative modes were analyzed simultaneously during a single sample injection, allowing all 26 mycotoxins to be analyzed in one data acquisition method. (Top) 18 mycotoxins were collected in ESI positive mode (top) and 8 mycotoxins were collected in ESI negative mode (bottom).

Results and Discussions

For each analyte, two signature MRM transitions were chosen to ensure confidence in the identification of each mycotoxin (Table 2). To monitor many MRM transitions during a single injection, the *Scheduled* MRM algorithm was employed, where individual MRM transitions were monitored for a short time window during their expected retention time. Thus, at any one point in time, the number of concurrent MRM transitions were significantly reduced resulting in much higher duty cycles for each analyte. Combining with fast polarity switching further allowed extending the target list of mycotoxins, thus maintaining sample throughput by eliminating need for multiple injections. Typical chromatograms of solvent standard were shown in Figure 2. The total target cycle time of 0.6 sec ensured the collection of at least 12 data points across the LC peak resulting in excellent accuracy and reproducibility. The system suitability was tested with the

concentration of 5/50 ng/mL standards and the standard solution was injected three times. The %CV of each analyte peak was calculated to less than 15%.

For sample preparation, a simplified sample clean-up method was developed. Instead of immunoaffinity columns, a special solid phase extraction (SPE) column (Cleanert® MC, Agela) with optimized sorbent chemistry for mycotoxin extraction was used. This column proved advantageous in that it doesn't need to be activated, washed, and eluted. It not only shortened the sample preparation time, but also saved cost. Figure 3 shows the comparison of the sample clean-up step before and after.

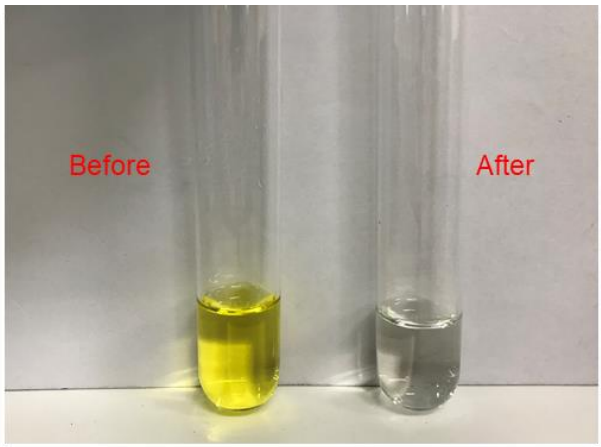

Figure 3. Sample Preparation and Clean Up. Visual comparison of a grain sample before and after the Cleanert® SPE column clean-up step. Cleaning up the sample can provide reduction of matrix interferences as well as help in maintaining instrument performance.

The limit of quantitation and matrix matched linearity were evaluated. Because of the matrix inhibitory effects, the matrix matched curves were used to quantify the unknown samples. For AflatoxinB1 (AFB1) and Deoxynivalenol (DON) as example, the method was found to have good reproducibility and the linear regression coefficient was found to be greater than 0.99 (Figure 4). According to the different sensitivity levels of each compound on the instrument, the LOQ of all targeted mycotoxins were from 0.5 ng/g to 20 ng/g. The accuracy of low, medium and high concentration spiked sample was between 80% and 120% (Figure 1).

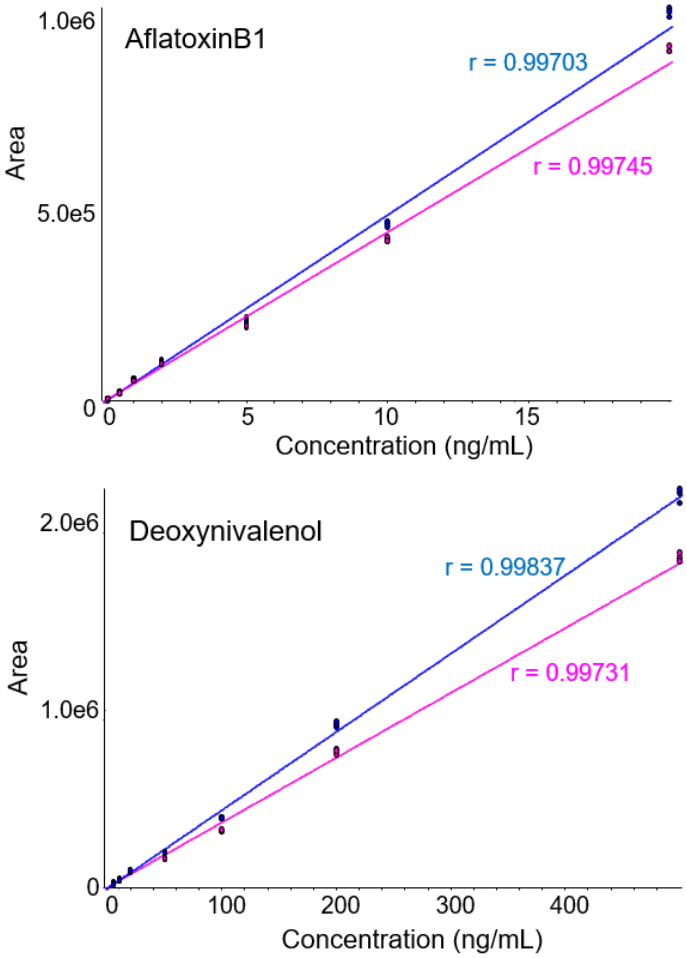

Figure 4. Calibration Curves for AflatoxinB1 and Deoxynivalenol. Calibration curves were generated from 5 to 500 ng/mL. Two MRM transitions were monitored: fragment 1 (blue) and fragment 2 (pink). R-values shown for both transitions for both representative analytes are >0.99, demonstrating excellent linear range and response for the assay.

Conclusions

A fast, robust, and reliable method for the detection 26 mycotoxins in the matrix grain was developed and validated. A fast purification method was used to cover the 26 kinds of mycotoxins. High resolution LC using a small particle size column was combined with high sensitivity detection using a SCIEX Triple Quad™ 3500 LC-MS/MS system. Multiple Reaction Monitoring (MRM) was used because of its high selectivity and sensitivity. The *Scheduled* MRM algorithm used to obtain optimized dwell times and cycle times for best sensitivity and reproducibility. The method was validated in different grain matrices. Limits of Quantitation (LOQ) of all mycotoxins were found between 0.5µg/kg and 20µg/kg. All LOQ meet the requirements of the grain Industry standard.

Table 2. MRM Transitions and Retention Times are Provided for Two Transitions for each Mycotoxin in the 26 Analyte Panel.

Compounds name	RT (min)	MRM (Primary, Quantifier)	MRM (Secondary, Qualifier)
AflatoxinB1 (AFB1)	*6.62*	*313.1>285.1*	*313.1>241.1*
AflatoxinB2 (AFB2)	*6.43*	*315.1>287.1*	*315.1>259.1*
AflatoxinG1 (AFG1)	*6.22*	*329.1>243.2*	*329.1>214.9*
AflatoxinG2 (AFG2)	*6.05*	*331.1>245.1*	*331.1>189.1*
AflatoxinM1 (AFM1)	*6.07*	*329.0>273.1*	*329.0>268.9*
AflatoxinM2 (AFM2)	*5.86*	*331.1>273.1*	*331.1>285.1*
T-2 toxin (T-2)	*8.32*	*484.2>305.3*	*484.2>185.1*
Verruculogen (VER)	*9.84*	*534.3>392.3*	*534.3>191.1*
Neosolaniol (NEO)	*5.41*	*400.2>185.1*	*400.2>305.2*
Wortmannin (WOR)	*7.59*	*447.2>345.2*	*447.2>285.2*
Roquefortine C (RC)	*7.13*	*390.3>193.1*	*390.3>322.2*
Sterigmatocysin (STE)	*9.19*	*325.1>310.1*	*325.1>281.0*
Lysergol (LYS)	*4.80*	*255.3>240.2*	*255.3>197.2*
Diacetoxyscirpenol (DIA)	*6.70*	*384.2>307.2*	*384.2>105.1*
HT-2 Toxin (HT-2)	*7.59*	*442.1>263.1*	*442.1>215.0*
Deoxynivalenol (DON)	*4.76*	*296.9>249.1*	*296.9>231.1*
3-Acetyl Deoxynivalenol (3-AcDON)	*5.80*	*339.0>231.0*	*339.0>203.0*
15-Acetyl Deoxynivalenol (15-AcDON)	*5.80*	*339.1>321.3*	*339.1>137.2*
Deoxynivalenol-3-Glucoside (DON-3G)	*4.83*	*503.1>427.1*	*503.1>457.1*

References

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