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Quantitation of Aflatoxins in the Traditional Chinese Medicine Coix Seed by UPLC Coupled with Tandem Quadrupole MS

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APPLICATION BENEFITS

- Fast and simple method for the identification and quantitation of aflatoxins in the coix seed in positive ionization mode
- Low level of detection using the ACQUITY UPLC® I-Class System and Xevo® TQ-XS Tandem Quadrupole Mass Spectrometer
- Improved throughput and efficiency of separation while maintaining chromatographic performance with CORTECS® Columns
- Excellent injection reproducibility with less than 0.9% RSD in matrix

WATERS SOLUTIONS

ACQUITY UPLC I-Class System

Xevo TQ-XS Tandem Quadrupole MS

CORTECS UPLC® C_{18} Column (2.1 x 50 mm, 1.6 µm) (p/n 186007093)

AflaTest® Immunoaffinity Columns (VICAM®) (p/n G1010)

MassLynx® Mass Spectrometry Software with TargetLynx™ Application Manager

KEYWORDS

Mycotoxins, TQ-XS, Chinese Pharmacopeia, herbal medicine, UPLC-MS/MS Quantification

INTRODUCTION

Mycotoxins are toxic secondary metabolites of fungi that grow on crops pre- and post-harvest. The presence of these toxins at certain levels in crops poses health risks for consumers[1] leading regulators to set limits in order to minimize exposure to the public. Traditional Chinese Medicines (TCM) often consist of or contain cultivated products and therefore testing for toxic contaminants becomes necessary.

Coix seed, also known as *Coix lacryma-jobi*, semen coicis, Job's tears, and Yi-Yi-Ren (薏苡仁), is the seed of a tall grass native to tropical Asia. It is commonly consumed in Asia as foods, and often as a tea or drink. It is also often prescribed for its therapeutic properties to restore balance by removing or clearing excess heat and dampness. Treatments are given to restore circulation in organs (spleen, kidneys, lungs) through the mild promotion of diuresis. Recently, it has been shown that coix seed also has synergistic effects on inhibiting tumor growth when its administration accompanies other chemotherapy agents[2] and its extract is the subject of past and present clinical trials in the United States.

Due to their toxicities, aflatoxin B1, B2, G1, and G2 limits in TCM in China are regulated at 5 µg/kg for B1 and 10 µg/kg for B2, G1, and G2 combined, according to the 2015 edition of Chinese Pharmacopeia. UltraPerformance Liquid Chromatography (UPLC) coupled with sensitive tandem mass spectrometry (MS) enables fast and confident analysis on small sample amounts. These platforms are key to ensuring the quality of consumed medicinal natural products used for treatment of various ailments. In this application note, we show how to quantify aflatoxin B1, B2, G1, and G2 confidently and rapidly using the Xevo TQ-XS and VICAM affinity sample prep.

EXPERIMENTAL

Standard preparation and sample extraction:

The traditional Chinese medicine, coix seed, was purchased from six sources A–F; one sample was pre-ground, the other five were dried, whole seeds. The seeds were mechanically ground to a very fine powder in a table-top grinder for processing. The extraction and purification method was used in accordance with the Chinese Pharmacopoeia. Figure 1 shows the extraction workflow. Calibrators were prepared by serial dilution of analytes spiked into 50:50 MeOH/H_2O. Five microliters of purified sample was injected onto the LC-MS system for analysis. Table 1 lists the MRM transitions, collision energies, and cone voltages for each analyte.

Method conditions

LC Conditions

LC system:	ACQUITY UPLC I-Class FTN System
Column:	CORTECS UPLC® C_{18} 2.1 x 50 mm, 1.6 µm
Column temp.:	25 °C
Inj. volume:	5 µL
Flow rate:	0.450 mL/min
Mobile phase A:	H_2O with 10 mM ammonium acetate
Mobile phase B:	Methanol
Gradient:	35 to 85% B over 2 minutes, 85 to 100% B over 0.5 minutes 100 to 35% B over 1 minute

MS conditions

MS system:	Xevo TQ-XS MS
Ionization mode:	ESI+
Capillary voltage:	1 kV
Source temp.:	150 °C
Desolvation temp.:	550 °C
Cone gas flow:	250 L/hr
Desolvation gas:	1000 L/hr

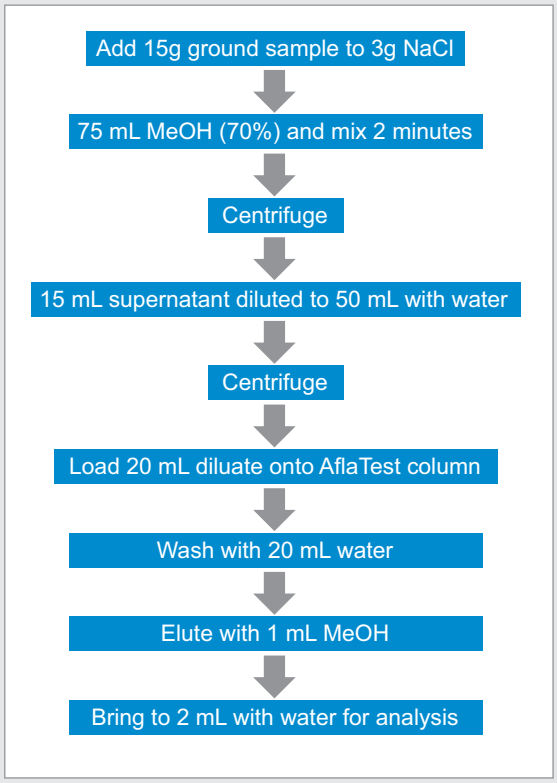

Figure 1. Sample preparation workflow.

	MRM transition	Cone voltage (V)	Collision energy (eV)	Dwell time (sec)	RT (min)
Aflatoxin B1 (AFB1)	Q 313>241 q 313>285	30	40 22	0.038	1.74
Aflatoxin B2 (AFB2)	q 315>259 Q 315>287	30	26 26	0.038	1.58
Aflatoxin G1 (AFG1)	Q 329>243 q 329>311	45	26 22	0.038	1.39
Aflatoxin G2 (AFG2)	Q 331>245 q 331>313	45	28 24	0.038	1.15

Table 1. MS/MS conditions used in the assay as obtained by optimization through IntelliStart.™ Transitions in bold text are quantifying traces while standard text are qualifying.

RESULTS AND DISCUSSION

RECOVERY, MATRIX FACTOR, AND METHOD SENSITIVITY/LINEARITY

The method for aflatoxins in Traditional Chinese Medicines as written in the Chinese Pharmacopoeia uses immunoaffinity purification in the sample preparation.[3] Extraction efficiency was tested by spiking 30 µL of 2.5 ng/µL into 15 g of ground coix seed to make 5 µg/kg, the regulatory limit. The measured concentration against the calibration curve for the final prepared sample was 3 ng/mL. The spiked sample was allowed to sit at room temperature in the dark for 60 minutes before extraction. Recovery was calculated against a sample spiked after extraction and matrix effects were calculated by comparing a spike after extraction sample to standard. Recovery and matrix factor are summarized in Table 2. Sensitivity and linearity were tested by preparing calibration curves of all four analytes in 50:50 MeOH/H_2O. AFB1, B2, and G1 had an LOQ of 0.0025 ng/mL (12.5 fg on column) and AFG2 had 0.005 ng/mL (25 fg on column) (Table 3). Each analyte exhibited 4 orders of linearity.

	Recovery*	Matrix factor**
AFB1	99	0.993
AFB2	101	1.02
AFG1	98	0.990
AFG2	66	1.23

Table 2. Recoveries and matrix factor for the analytes in coix seed, n=5.
** Recovery = extracted ÷ spike after extraction*
*** Matrix factor = spike after extraction ÷ standard*

	LLOQ (ng/mL)	HLOQ (ng/mL)	R^2
AFB1	0.0025	25	0.998
AFB2	0.0025	25	0.999
AFG1	0.0025	25	0.999
AFG2	0.0050	50	0.999

Table 3. Linear range of the analytes in solvent.

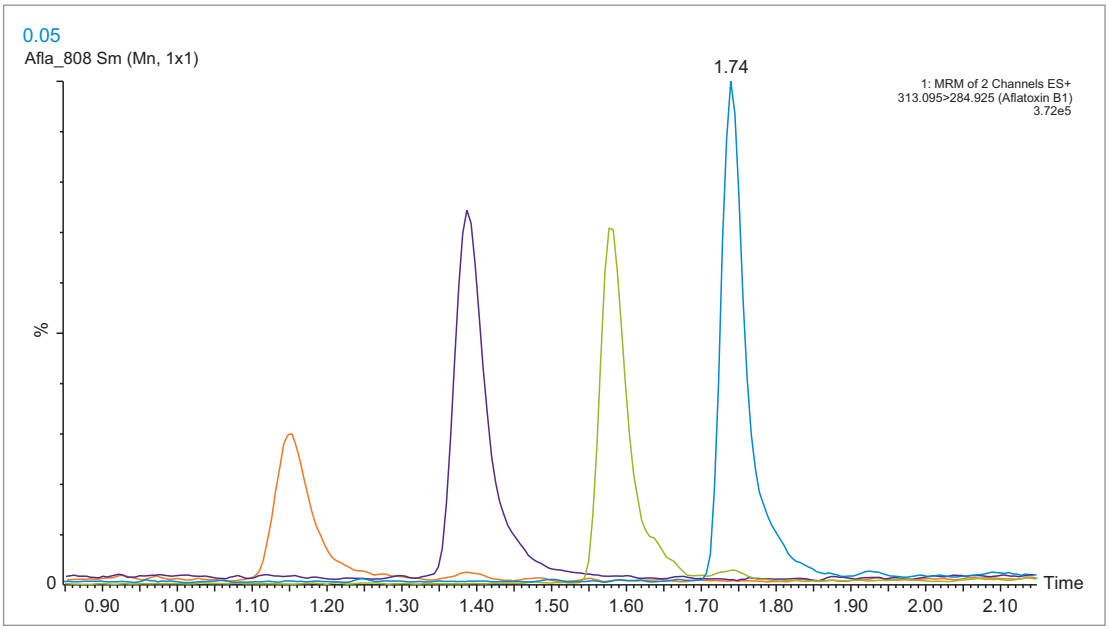

Figure 2. Representative separation of AFB1, AFB2, AFG1, and AFG2 in solvent.

METHOD ROBUSTNESS

To test the robustness of the assay in matrix, a series of injections was performed at the Chinese Pharmacopoeia regulatory limit of 5 µg/kg for the aflatoxins. Figure 3 shows an example TrendPlot of AFB1 over 200 injections. The RSD areas were 0.85%, 0.79%, 0.76% and 0.89% for AFB1, AFB2, AFG1, and AFG2 respectively indicating solid method robustness.

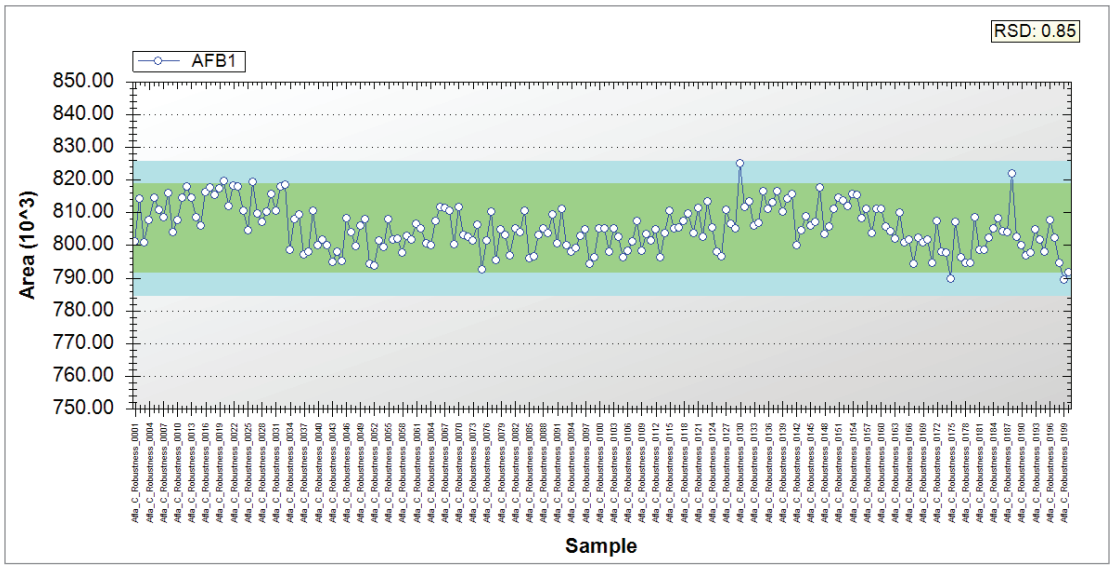

Figure 3. TrendPlot of AFB1 at 5 µg/kg over 200 injections in matrix. Green shading is 2σ and blue is 3σ.

SAMPLE ANALYSIS

Coix seed from 6 different suppliers was purchased and extracted as described above. A system suitability sample at 1 ng/mL was prepared by diluting an appropriate volume of 25 µg/mL stock solution of the four aflatoxins into 50:50 MeOH/H_2O. The retention time and ion ratios were referenced from the system suitability injections. Concentrations in µg/kg were calculated from a solvent calibration curve using the following equation:

X = (A*2*50*75) ÷ (20*15*m) where X is µg/kg in coix seed, A is calculated concentration in ng/mL, and m is the mass weighed of the sample. The TargetLynx XS quantitation results are shown for AFB1 in figure 4 (TargetLynx Application Manager automates sample data acquisition, processing, and reporting for quantitative results.)

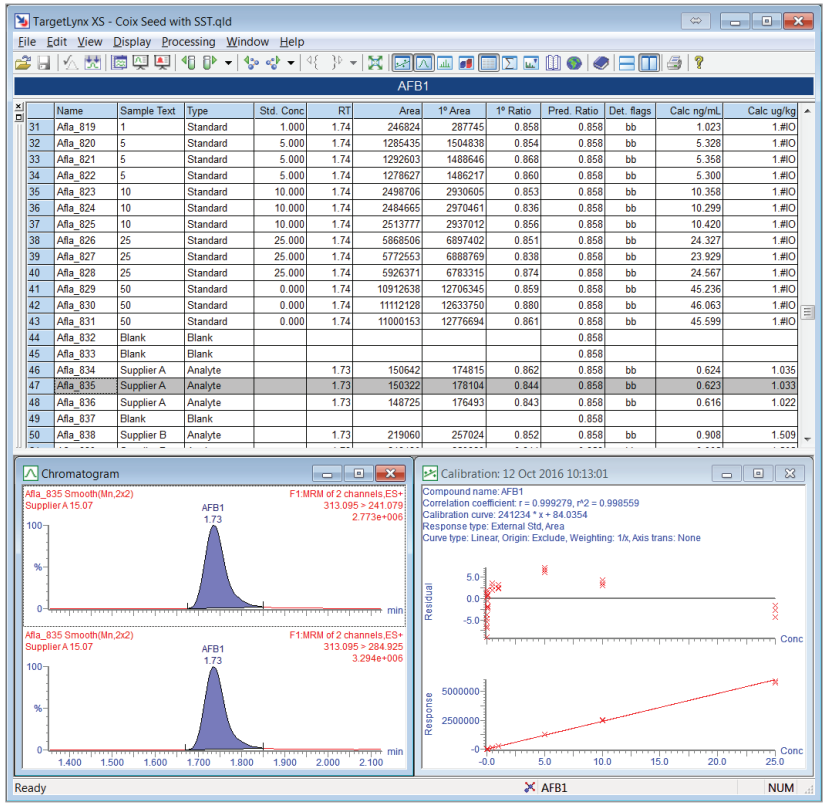

Figure 4. TargetLynx results for AFB1.

A summary of the concentrations of aflatoxins in coix seed are listed in table 4. As is listed, AFB1 is found in every sample at varying concentration levels with the other aflatoxins at much less concentration, all below the regulatory limits of 5 µg/kg for AFB1 and 10 µg/kg for AFB2, AFG1, and AFG2 combined.

	AFB1	AFB2	AFG1	AFG2	Sum AFB2, AFG1, AFG2
Supplier A	1.03	0.063	0.014	n/d	0.020
Supplier B	1.51	0.088	n/d	n/d	0.088
Supplier C	0.037	BLOQ	n/d	n/d	–
Supplier D	0.199	0.016	0.007	n/d	0.023
Supplier E	0.075	0.006	0.008	n/d	0.014
Supplier F	0.029	BLOQ	n/d	n/d	–

Table 4. Shows summary of mean aflatoxin concentration in µg/kg as calculated from a solvent calibration curve, n=3.

CONCLUSIONS

- A UPLC-MS/MS method for the quantitation of aflatoxins had been developed and applied to the Traditional Chinese Medicine coix seed from different suppliers
- The linearity and robustness of the Xevo TQ-XS and the sensitivity it affords ensures new analytical methods can achieve high sensitivity to future-proof your lab for challenging assays
- Combining VICAM solutions with high performance tandem quadrupole detection enables rapid and accurate testing of aflatoxins in natural products
- Single platform and workflow solution allows plug and play high sensitivity and accurate analysis of aflatoxins using the current regulation methodology

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