Analysis and Quantification of Mycotoxins in Cereals Using MRM^{HR} on the SCIEX X500R QTOF System with SCIEX OS

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Introduction

Mycotoxins are toxic secondary metabolites produced by fungi under favorable conditions which can cause acute and chronic illness in humans and animals [1]. The most common mycotoxins that threaten human health include aflatoxin (AF), deoxynivalenol (DON), ochratoxin A (OTA), fumonisin (FB), zearalenone(ZEN), and T-2 toxin(T2) [2], which are widely found in many grains and oils, and their products. To date, many countries and regions have set strict mycotoxin limits [3], and China has revised its Food Safety Law in 2015 to explicitly include biotoxins in contaminants of major concern for the first time. China has also set limits for mycotoxins such as deoxynivalenol (DON), zearalenone (ZEN), aflatoxin B1(AFB1), and ochratoxin (OTA); for instance, the state food safety standard for cereals set the limit for ZEN at 60μg/kg, aflatoxin B1(AFB1) at 5-20μg/kg, and OTA at 5μg/kg[4].

Abnormal climate conditions and insect damage have caused extensive mycotoxin contamination of grain in recent years, leading to severe damage and huge losses. Reports show that in China, 31 million tons of grain are contaminated with mycotoxins annually during production, storage, and transport; this accounts for approximately 6.2% of total grain production. Rapid, high-throughput, and highly accurate mycotoxin detection methods with simple sample pretreatment are critical to ensuring the quality and safety of China's grain supplies, strengthening the monitoring for mycotoxins in grain, as well as protecting human and animal health.

The X500R QTOF system combines high mass resolution and mass accuracy with quantitative capabilities. As with triple quadrupole- mass spectrometry, it is possible to collect multiple reaction monitoring (MRM) ion pair data; however, the QTOF can obtain high-resolution MSMS ions with ultra-rapid scanning speeds (100Hz) in a mode called MRM^{HR} scanning mode [Fig 2]. This scanning mode offers high selectivity in the same manner as quadrupole devices and provides quantitative analysis through high-resolution secondary product ion peak areas and qualitative analysis through the calculation of ion abundance ratios. This capability helps to avoid false positives or other matrix effects due to interferences.

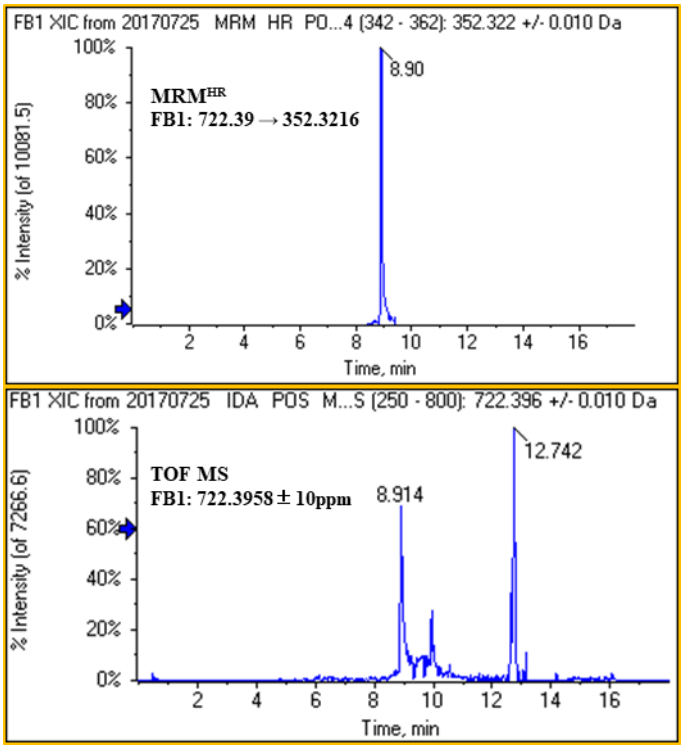

Figure 1. Scheduled MRM^{HR} selectivity compared to TOFMS for FB1. Increased selectivity is demonstrated by monitoring the ion transition using MRM^{HR} compared to monitoring solely the target precursor ion and reduces the potential for false negatives or incorrect integration of matrix peaks.

Key Advantages: MRM^{HR} for Mycotoxins

- Sample pretreatment is simple and fast, only 10 minutes from sample preparation to testing
- Fifteen common mycotoxins are included in the panel; because mycotoxin reference products are expensive and difficult to obtain, this study produced high-resolution MS/MS spectral data allowing identification without reference products
- Scheduled MRM^{HR} scanning mode, retention time locking, and established MRM^{HR} ion parameters greatly reduce matrix effects and increase method reproducibility and accuracy. The method is easily applied, saving on development time and costs.

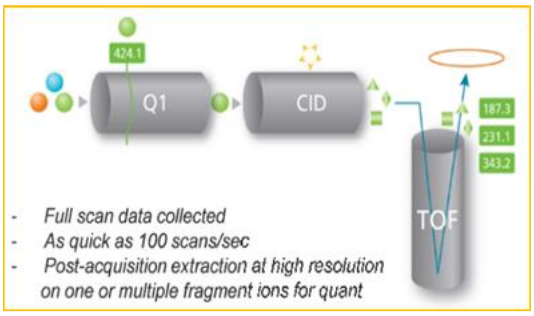

Figure 2. High Resolution MRM (MRM^{HR}) ion path.

Experimental methods

Table 1. Chromatography Conditions

LC Column	Shim-pack XR ODS, 2.0x 75mm 1.6μm
Mobile Phase A	*Water with 2mM ammonium acetate and 0.1% Formic Acid*
Mobile Phase B	*Pure acetonitrile*
Flow Rate	*0.3mL/min*
Column Temperature	*40°C*
Injection Volume	*2μL*

Table 2. Mass Spectrometry Conditions

Scanning Method	*Scanning range 100 – 1000 Da Scheduled MRM^{HR}*		
CUR	30 psi	*CAD*	*7 psi*
IS Voltage	*5500V / -4500V*	*TEM*	*550°C*
GAS 1	*55 psi*	*GAS 2*	*50 psi*

Sample Preparation Method

1. •Take a 0.5g crushed wheat sample
2. •Add 2 mL acetonitrile-water-acetic acid (70/29/1); shake for 1min
3. •Centrifuge at 12000r/min for 5min
4. •Dissolve the supernatant in water by 1-fold;
5. •Pass through a 0.22 μm filter for testing;

This sample preparation method is simple, fast, and is suitable for wheat, corn, rice, and sesame; a total of 10 grain samples were collected from different production regions.

Compound information

Compound	CAS no.	Formula
Aflatoxin B1 AFB1	001162-65-8	C17H12O6
Aflatoxin B2 AFB2	007220-91-7	C17H14O6
Aflatoxin G1 AFG1	001165-39-5	C17H12O7
Aflatoxin G2 AFG2	007241-98-7	C17H14O7
Ochratoxin A OTA	000303-47-9	C20H18ClNO6
T-2 toxin T-2	021259-20-1	C24H34O9
Fumonisin B1 FB1	116355-83-0	C34H59NO15
Fumonisin B2 FB2	116355-84-1	C34H59NO14
Fumonisin B3 FB3	136379-59-4	C34H59NO14
Aflatoxin M1 AFM1	6795-23-9	C17H12O7
Nivalenol NIV	023282-20-4	C15H20O7
Deoxynivalenol DON	051481-10-8	C15H20O6
3-Acetyl-deoxynivalenol 3-AcDON	876926-22-6	C17H22O7
15-Acetyl-deoxynivalenol 15-AcDON	088337-96-6	C17H22O7
Zearalenone ZEN	017924-92-4	C18H22O5

X500R SCIEX OS MRM^{HR} Work Flow

The quantification capabilities of the X500R QTOF high-resolution mass spectrometer are comparable to those of the triple quadrupole MRM device; however, the SCIEX OS platform design includes a unique MRM^{HR} work flow. User defined MRM ion pair information can be entered similarly to quadrupole devices in the SCIEX OS software [Fig 3]. The precursor ion

window is set to unit resolution, as in quadrupole mode, and the product ions are separated using a TOF mass filter; thus high-resolution product ions are produced [Fig 1]. MRM^{HR} ion pair data can be imported directly from high-resolution secondary libraries into MRM^{HR} method lists [Fig. 3]. Data for 5 ion pairs from each compound can be imported based on the sensitivity ranking of the secondary product ion in the library. MRM^{HR} lists with imported data include retention time, as well as unique voltage parameters for each ion pair such as declustering potential (DP) and collision energy (CE).

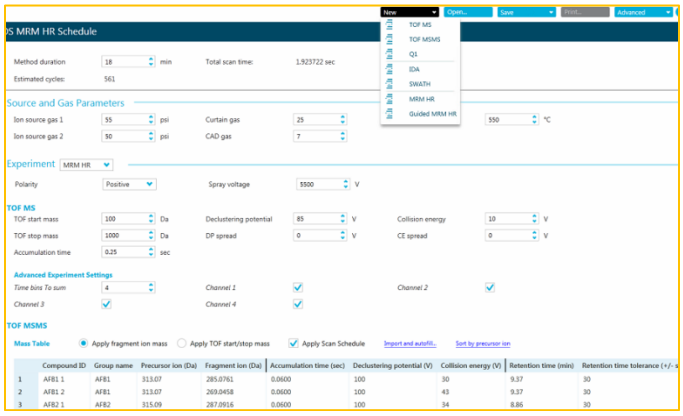

Fig 3. SCIEX OS Software Scheduled MRMHR Method Editor, Applying Fragment Ion Mass ±10ppm m/z

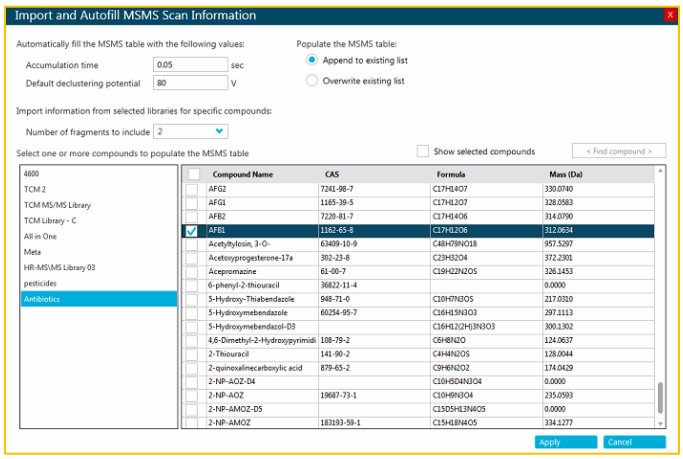

Fig 4. Import and Autofill MSMS Scan Information

Experimental results

1. Mobile phase optimization

Comparison of the influence of mobile phases with varying ratios of modifier on ionization of various mycotoxins shows that ammonium acetate causes a more obvious signal increase than ammonium formate, while a lower salt concentration (2mmol/L ammonium acetate) has a stronger effect than higher concentrations (5mmol/L and 10mmol/L). FB1 and FB2 show a strong effect on signal production when acid (0.1%) is added. Ultimately, a weak aqueous eluent containing 0.1% formic acid and 2mmol/L ammonium acetate was selected as the mobile phase A.

2. Target ion selection

This study optimizes the effects of adduct ions of various toxins in a detailed manner. For example, T-2 toxin has peaks for hydrogen, ammonium, and sodium adducts; given that the sodium peak is not fragile, this study compared hydrogen and ammonium adduct peaks and found that the ammonium peak signal was 30 times that of the protonated peak [Fig 5]. In addition, the effects of formic acid adducts in NIV and DON were greater than those of dehydrogenation peaks [Fig 6].

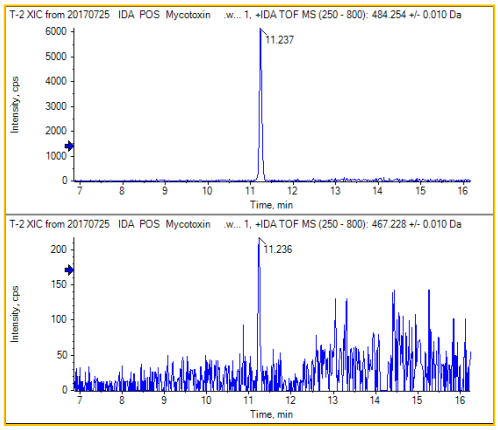

Fig 5. Chromatogram of T-2: ammonium adduct (top) produced a greater absolute signal versus protonated precursor (bottom).

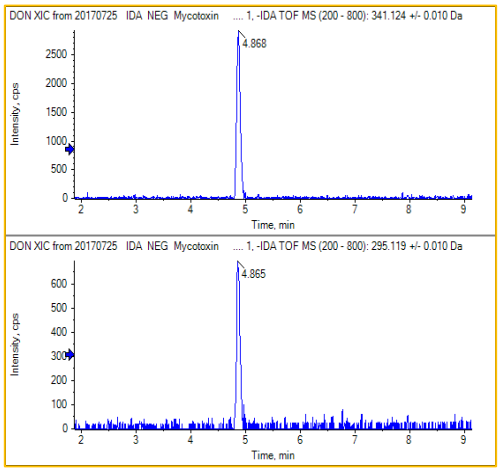

Fig 6. Chromatogram of DON: formate adduct (top) produced greater absolute signal than dehydrogenated precursor (bottom).

3. Strong resistance to matrix interference

Sample preparation involved a simple liquid-liquid extraction and dilution, which does not remove much background sample matrix and can therefore leave the sample subject to interference effects. Four grain matrices were assessed for matrix effects based on the ratio of the areas of the target in the blank solvent and the matrix solvent. As the ratio approached 100%, matrix effects became insignificant; above 100% matrix effects were considered “enhancement”; below 100% there was matrix suppression. Matrix effects of high-resolution primary scanning and MRM^{HR} scanning were also compared. **When TOFMS scanning of 15 mycotoxins was performed, matrix effects ranged between 43.1% and 125.6%, and when MRM^{HR} scanning was performed, matrix effects ranged between 88.5% and 109.2%.** The results showed that MRM^{HR} scanning is much more selective and resistant to matrix interference than primary TOFMS scanning. Figures 1 and 6 show that after the addition of corn matrix to fumonisin B1, there was strong interference with the TOF-MS primary scan, while the MRM^{HR} scan showed less noise and greater selectivity. Aflatoxin AFB1 is highly sensitive, and matrix inhibition effects are minimal when TOF-MS scanning is performed. Thus, this study shows that MRM^{HR} scanning is more selective and resistant to matrix inhibition, increasing method accuracy and reproducibility, which can in turn greatly decrease positive and false negative results.

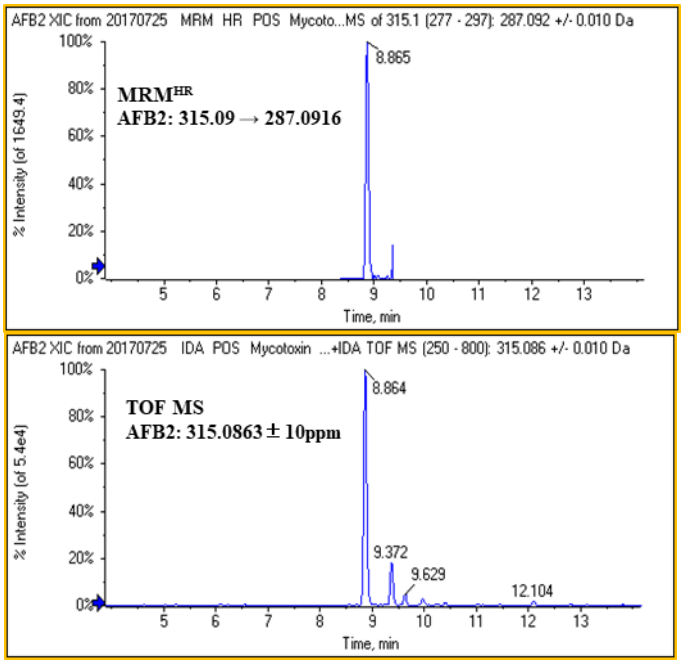

Fig 6. Scheduled MRMHR selectivity for AFB2 compared to TOFMS; increased selectivity with MRMHR reduces the potential for false negatives or incorrect integration of different peaks.

4. Simultaneous quantification and qualification

At concentrations between 0.05 and 50ng/mL, there were strong linear responses for all mycotoxins [Fig 7]; the correlation coefficients were greater than 0.99, fully meeting quantitative analysis requirements.

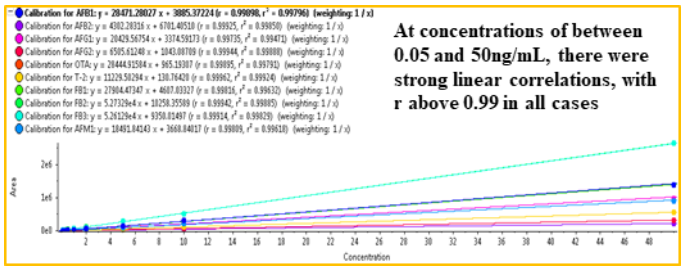

Fig 7. The Regression Calibrations of Mycotoxins

SCIEX OS software calculates ion abundance ratios in a similar manner to quadrupole devices, and different compounds have qualitative and quantitative differences in terms of ion abundance ratios. International and EU regulations [Table 3] can be used to determine the range of ratios.

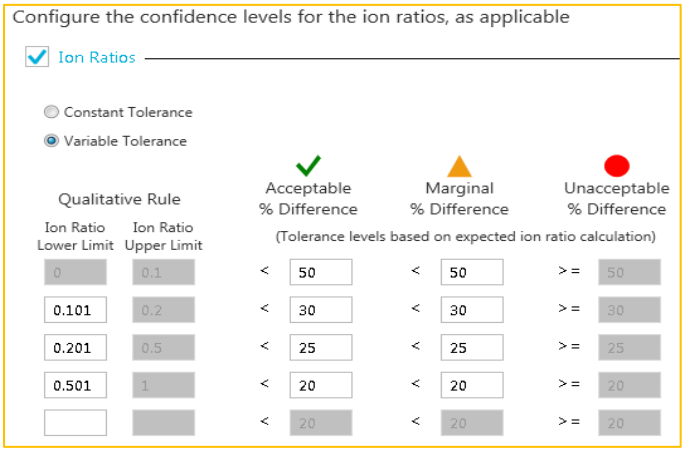

Fig 8. The MRM^{HR} Ratio tolerance limits can be user-defined in the SCIEX OS software

Table 3. EU regulations from the SANTE/11945/2015 document

MS detector / characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[e] Analyte peaks in the extracted ion chromatograms must fully overlap. Ion ratio within **±30% (relative)** of average of calibration standards from same sequence
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a,b,c]	
		combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions: 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm[a,c] plus 1 MS/MS product ion[d]	

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] no specific requirement for mass accuracy
[e] in case noise is absent, a signal should be present in at least 5 subsequent scans

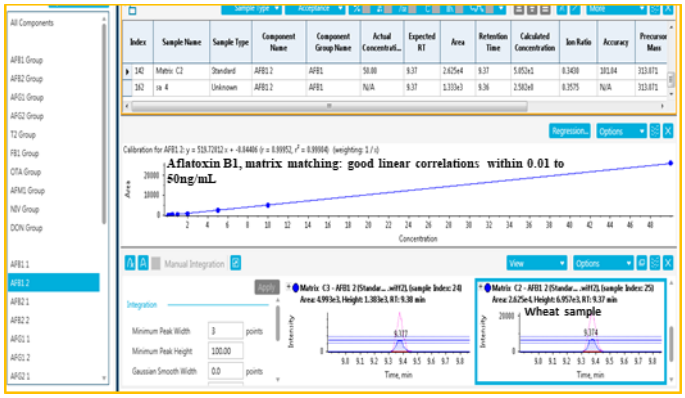

Fig 9. Quantification results for AFB1 in wheat as they appear in the SCIEX OS software user interface

When data of actual samples were analyzed, as shown in Fig 9, they were processed quickly and easily, and the interface was intuitive. The result showed a standard curve, a sample and matrix spiked ion abundance ratio, accuracy, sample concentration, and retention time. One can intuitively determine that wheat produced in a certain area contains aflatoxin B1 (AFB1) based on a sample ion abundance ratio of 0.357, a matrix spiked ion abundance ratio of 0.343, and concentration of 2.5µg/kg. This study examined 10 samples including wheat, corn, etc., from various provinces. See Table 3 for the detection results.

Table 4. Mycotoxin content of wheat (n=6) and corn (n=4) from different regions. A blank space in the table indicates not detected.

	Wheat						Corn			
µg/kg	1	2	3	4	5	6	1	2	3	4
AFB1	1.6	1.1	0.6	2.5	2.1	1.9	2.2	6.2	2.1	0.8
AFB2				0.2		0.5		0.9		
AFG1										
AFG2										
OTA										
T-2										
DON	331.1	498.8	210.2	112.2	99.9	120.2	98.9	76.9	88.1	120.3
FB1										
FB2										
FB3										
AFM1										
NIV	150.1	122.3	144.5	112.6	90.3	160.4				
3-AcDON	13.5	10.6	9.9	13.2	40.2	26.0	16.8	19.2	10.3	11.5
15-AcDON	19.9	11.0	13.4	16.6	54.2	31.9	15.9	18.3	21.9	22.1
ZEN	20.1	15.6	4.8	8.9	9.9	2.7	2.8	4.5	0.9	6.5

Summary

This report describes the use of the novel SCIEX X500R QTOF system to establish techniques for the detection of common mycotoxins in grain samples. The MRM^{HR} scanning capability offers the high sensitivity of existing quadrupole devices as well as high-resolution quantitative product ion detection (peak area) and qualitative confirmation (ion abundance ratios) at ultra-rapid scanning speeds. Because this capability is selective and resistant to matrix interference, sample preparation techniques were able to be further simplified, decreasing labor-intensive steps and improving work efficiency. Since mycotoxin reference samples are expensive and difficult to obtain, a high-resolution secondary library of mycotoxins was created. This removes the need for reference samples and permits easy identification of mycotoxin species in a sample. In summary, this method is

sensitive, meets international and EU requirements for mycotoxin limits, and fully complies with customer analytical requirements. It can serve as a valuable reference in the application of high-resolution instrumentation to the detection of mycotoxins in grain samples.

This study included a total of 10 samples of wheat and corn from different production regions. Testing showed AFB1, DON, NIV, ZEN, 3-AcDON and 15-AcDON were widely distributed throughout the wheat and corn sample, illustrating the prevalence of mycotoxin contamination of grain samples and highlighting the need for greater monitoring and oversight.

References

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2. Sulyok M, Berthiller F, Kraska R, et al. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize [J] Rapid Commun Mass Spectrom, 2006, 20(18): 2649-2659

3. Commission Regulation (EC) No 1881/2006, Setting Maximum Level for Certain Contaminants in Food Stuffs

4. GB2761-2011, People's Republic of China State Standard for Mycotoxin Limits in Food Products

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