The role of analytical technologies in detecting mycotoxin and tropane alkaloid food contamination amidst climate change

Holly Lee, PhD, Staff Scientist, Food Market, SCIEX

As climate change continues to reshape our world, its impact on food safety is becoming a growing concern. One of the most pressing issues in agriculture and food production is the increased prevalence of mycotoxin contamination. Mycotoxins are toxic secondary metabolites produced by fungi, particularly species of *Aspergillus*, *Fusarium*, and *Penicillium*, which thrive in warm and humid conditions.[1] Rising global temperatures and shifting precipitation patterns have created an environment that fosters fungal growth, leading to increased occurrences of mycotoxin contamination in crops.

These contaminants pose serious health risks, including carcinogenic, nephrotoxic, hepatotoxic, and immunosuppressive effects. In response, the food industry and regulatory agencies must strengthen food safety testing measures to mitigate risks associated with mycotoxins. Moreover, with climate change facilitating the spread of new plant pathogens, farmers are compelled to adjust their pesticide strategies, either by increasing application frequency or adopting novel pesticide formulations. This creates an additional concern: plants are often weakened by periods of extreme weather, making them more susceptible to pests and diseases and necessitating increased pesticide use.[2] At the same time, mycotoxin-producing fungi are flourishing under these altered environmental conditions.

Beyond mycotoxins, another growing concern in food safety is the presence of tropane alkaloids, such as scopolamine and hyoscyamine. These naturally occurring toxins, found in certain invasive plant species such as *Datura stramonium* (Jimson weed), pose serious health risks when inadvertently co-harvested with food crops.[3] The increase in erratic climate patterns has led to the unintentional spread of tropane alkaloid-containing plants into agricultural fields, raising the risk of contamination in grain and seed-based products. Addressing these risks requires advanced analytical techniques that can effectively detect and quantify both mycotoxins and tropane alkaloids in food matrices.

Furthermore, masked mycotoxins - modified forms of mycotoxins that escape conventional detection methods like immunoassay-based techniques - are emerging as a critical challenge.[1,4] These metabolites are transformed by plants or microorganisms and can revert to their toxic parent compounds during digestion, posing a hidden health risk. Advanced analytical techniques like mass spectrometry (MS) enable targeted and nontargeted screening to achieve comprehensive coverage of both these compounds and their parent analogues, while also providing the sensitivity required for their quantitation.

Regulatory agencies, such as the European Union (EU), Food and Agriculture Organization (FAO), and the World Health Organization (WHO), have imposed strict maximum residue levels (MRLs) to ensure consumer safety, particularly for baby foods and staple grains.[5,6] Key regulated mycotoxins include aflatoxins (e.g., AFB1), which are highly carcinogenic, ochratoxins (OTA), patulin, fumonisins (e.g., FB1, FB2), zearalenone (ZEN), deoxynivalenol (DON), ergot sclerotia and alkaloids, T-2 and HT-2 toxins, and trichothecenes.[5]

Ultra-sensitive and selective detection methods are necessary to accurately identify and quantify mycotoxins, masked mycotoxins, and tropane alkaloids in complex food matrices at levels that are compliant with those established by regulatory agencies. These analytical methods include advanced applications, workflows, and instruments, with cutting-edge MS leading the way.

Analytical approaches to detect mycotoxins and tropane alkaloids

The selection of an appropriate method depends on factors, such as sensitivity, specificity, throughput, cost, and regulatory compliance. Conventional approaches have distinct advantages and inherent limitations. These include bioanalytical methods that rely on immuno-recognition or receptor binding, such as enzyme-linked immunosorbent assay (ELISA), dipsticks, lateral flow devices, and immuno-sensors. Immunoassay-based techniques, like ELISA and lateral flow assays, are cost-effective, rapid, and ideal for high-throughput and field applications. They enable on-site food testing but are prone to cross-reactivity, which may lead to false positives. Their inability to differentiate between masked and free mycotoxins also limits their effectiveness, making them more suitable for preliminary rapid screening.[7,8]

Other techniques, such as thin-layer chromatography (TLC), may also be used, provided that the signals they generate directly correspond to the mycotoxins of interest.[5,6] As a low-cost and easy-to-use technique, TLC is valuable in resource-limited settings for initial screenings.

However, its low sensitivity, semi-quantitative nature, and time-consuming process make it unsuitable for regulatory routine testing without confirmatory analysis.[7,9] Advancements in miniaturised MS and biosensors enable real-time, onsite monitoring. Handheld devices utilising near-infrared and Raman spectroscopy provide non-destructive detection of mycotoxins and tropane alkaloids, enhancing food safety interventions.[10,11,12,13]

Chromatographic approaches, such as high-performance liquid chromatography (HPLC) and gas chromatography (GC), are often integrated with detection methods like fluorescence detection (FLD) and mass spectrometry (MS and MS/MS) to enhance orthogonal separation of different compounds. HPLC-FLD reliably quantifies mycotoxins with high sensitivity but is limited to fluorescent analytes. Its limited detection of masked mycotoxins and tropane alkaloids, along with the need for complex derivatisation, adds to its drawbacks.[7,9]

Ambient MS enables rapid and onsite screening of food contaminants with minimal preparation. However, it lacks the sensitivity and quantitative performance of LC-MS/MS and may be prone to matrix interferences.[7,10] GC-MS excels in detecting volatile and thermally stable compounds, particularly tropane alkaloids. However, it requires derivatisation for non-volatile mycotoxins, has longer analysis times, and is less sensitive to polar mycotoxins.[7,9] Regarded as the gold standard for mycotoxin quantitation, LC-MS/MS offers high specificity, sensitivity, and multi-residue detection capability, but high costs and the requirements of highly trained personnel and complex sample preparation may present challenges for widespread adoption.[7,11]

The advantages of the latest mass spectrometry technologies

Effective chromatographic separation is crucial for the accurate quantification of mycotoxins through LC-MS/MS analysis. HPLC helps ensure that structurally similar mycotoxins are well separated *(see Figure 1)*. Advanced workflows in toxin analysis integrate streamlined approaches, such as the Quick Easy Cheap Effective Rugged Safe (QuEChERS) sample preparation protocol, solid-phase extraction (SPE) purification technique, and optimised gradient elution methods.[11] These approaches help minimise matrix interferences and enhance analyte retention and peak resolution, leading to more accurate quantitation of mycotoxins and tropane alkaloids. Additionally, optimised targeted workflows, such as multiple reaction monitoring (MRM), improves specificity by further minimising matrix interferences, resulting in high signal-to noise ratios.

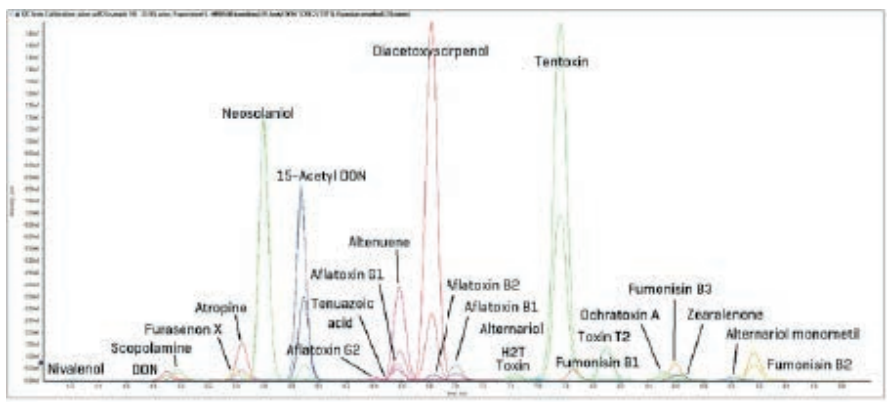

Figure 1: Extracted ion chromatogram (XIC) showing chromatographic separation of mycotoxins in a wine matrix, with all tested mycotoxin compounds overlaid, and using SCIEX OS 3.4 software for data processing, including the peak-to-peak signal-to-noise and MQ4 integration algorithm.[11] Credit: SCIEX.

These approaches continue to be refined in the latest LC-MS/MS technologies. For instance, a new triple quadrupole mass spectrometer has been engineered to maintain the optimal sensitivity performance for up to twice as long as compared with existing SCIEX technology, particularly when running complex matrices.[14,15] The SCIEX 7500+ system is the fastest scanning SCIEX triple quadrupole system so far and can acquire up to 800 MRM transitions per cycle, increasing the scope for large quantitation panels that incorporate new compounds of concern.[14] An LC-MS/MS analysis using a simplified QuEChERS protocol and MRM quantitation on the SCIEX 7500+ system achieved LOQs for all the mycotoxins and tropane alkaloids tested that were 10–250 times lower than the lowest MRLs required by EU legislation.[11] The LOQs for mycotoxins such as aflatoxins and ochratoxin A in cereal-based products were in the sub-parts per billion (ppb) range, well below the regulatory limits set for these matrices.[5,11] Similarly, in dairy products and infant food, where MRLs were stricter, the SCIEX 7500+ system achieved LOQs well below these regulatory thresholds *(see Figure 2)*.[5,11] The high sensitivity of the MS system enabled a small injection volume of 1.5 µL, while still capable of sub-ppb detection, resulting in low carry-over. The method demonstrated accurate and highly reproducible quantitative performance for all compounds in baby food, almond, grape juice, and wine matrices.

The system has demonstrated increased resilience in analysing a large range of sample types and workflows, under the most extreme conditions.[14] This is partly due to the inclusion of an innovative proprietary technology, Mass Guard technology, which actively filters out potentially contaminating ions. This reduces the risk and frequency of instrument contamination.[15,16] Another refinement is the newly designed DJet+ assembly, which is fully removable and can be cleaned and maintained by users, making it easier to schedule front-end cleaning and maximise system uptime.[14,15]

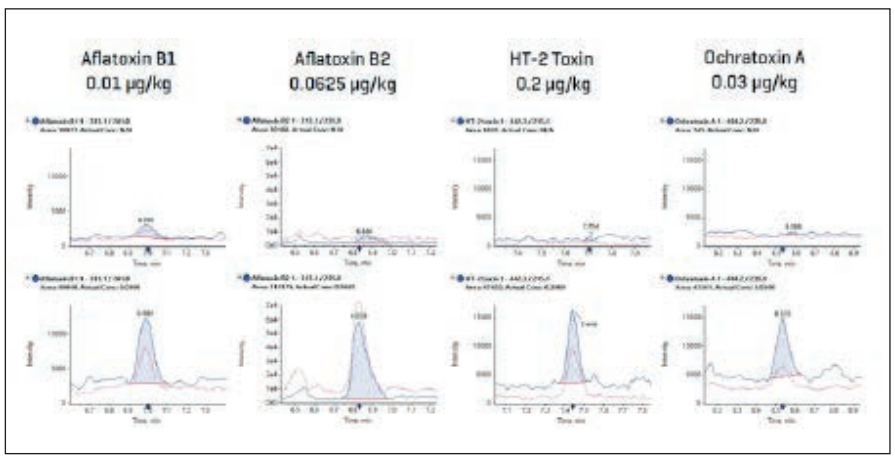

Figure 2: **A***: XICs of four mycotoxins in the matrix blank (first row) and in baby food pre-spiked at the LOQ. Quantifier ion is shown in blue, first qualifier ion is shown in pink. Using the SCIEX 7500+ system, LOQ values of 0.01 µg/kg were achieved for aflatoxin, 0.0625 µg/kg for aflatoxin B2, 0.2 µg/kg for HT-2 toxin, and 0.03 µg/kg for ochratoxin A. These values are 10–250 times lower than the MRL values required in regulation EC 2023/915 for these specific mycotoxins in baby food matrix.[5,11]*

Component	Lowest EU MRL (µg/kg)[1,2,3]	LOQ, baby food (µg/kg)	LOQ, almond (µg/kg)	LOQ, grape juice (µg/kg)	LOQ, wine (µg/kg)
15-Acetyl DON	-	2.0	1.0	1.0	1.0
3-Acetyl DON	-	2.0	1.0	1.0	1.0
Aflatoxin B1	0.1	0.01	0.01	0.01	0.01
Aflatoxin B2	4.0 (sum B1, B2, G1, G2)	0.0625	0.0625	0.005	0.005
Aflatoxin G1	4.0 (sum B1, B2, G1, G2)	0.01	0.01	0.01	0.01
Aflatoxin G2	4.0 (sum B1, B2, G1, G2)	0.125	0.0625	0.005	0.005
Altenuene	-	0.4	0.2	0.2	0.2
Alternariol	2.0	0.04	0.04	0.1	0.1
Alternariol monomethyl	2.0	0.04	0.04	0.02	0.02
Atropine	0.2 (sum of atropine and scopolamine)	0.1	0.1	0.02	0.02
Diacetoxyscirpenol	-	0.4	0.4	0.2	0.2
DON	200	1.0	1.0	0.2	0.2
Fumonisin B1	100.0 (sum of B1 and B2)	0.2	0.2	0.2	0.2
Fumonisin B2	100.0 (sum of B1 and B2)	0.2	0.2	0.2	0.2
Fumonisin B3	-	0.2	0.2	0.2	0.2
Fusarenone X	-	2.0	1.0	0.4	0.4
HT-2 toxin	10.0 (sum of HT-2 and T2)	0.2	0.04	0.04	0.04
Neosolaniol	-	0.2	0.2	0.2	0.2
Nivalenol	-	1.0	0.4	1.0	0.4
Ochratoxin A	0.5	0.03	0.012	0.012	0.012
Scopolamine	0.2 (sum of atropine and scopolamine)	0.05	0.02	0.01	0.01
Tentoxin	-	0.2	0.2	0.2	0.2
Tenuazoic acid	100	100	100	2.0	2.0
Toxin T-2	10.0 (sum of HT-2 and T2)	0.08	0.04	0.04	0.04
Zearalenone	20	0.1	0.1	0.1	0.1

Figure 2: **B***: LOQ values achieved for mycotoxins in 4 food matrices using standard MRM workflow, compared with the lowest MRL value required by Regulation EU 2023/9151, EU 2024/10382 and Recommendation EU 2022/5533.[11] Credit: SCIEX.*

LC-MS/MS offers the benefit of comprehensive multi-residue screening. Contamination often involves the co-occurrence of multiple toxins and other endogenous components in a single sample, which necessitates an analytical approach that can simultaneously quantify various contaminants and resolve them from matrix interferences.

The SCIEX 7500+ system is enabled with QTRAP technology, which combines the capabilities of a triple quadrupole MS with a linear ion trap (LIT) for simultaneous quantitation and qualitative identification.[11,18] For example, as part of the QTRAP functionality, the MRM^3 workflow provides enhanced specificity for analyte detection in complex matrices like baby food *(see Figure 3)*. The dual fragmentation of analyte precursor ions results in the production of first- and second-generation product ions, yielding more unique and compound-specific MRM^3 transitions for monitoring. This increased specificity enables the removal of co-eluting matrix interferences, which can improve signal-to-noise and LOQ selection due to a cleaner chromatographic background.[11,18]

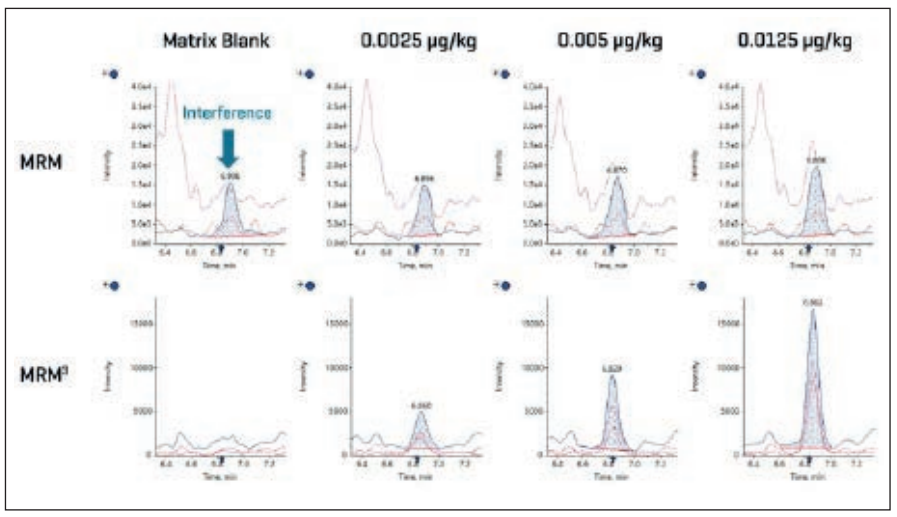

Figure 3: Comparison of MRM and MRM3 scan XICs of blank and low-level standards for aflatoxin B2 in baby food matrix. The XICs show that the MRM scan for aflatoxin B2 in baby food contains an interference, indicated in the matrix blank, and results in accurate quantitation not being possible below 0.0625 µg/kg. MRM^3 provides an alternative solution when MRM-based quantitation is hindered by high background and co-eluting interferences. The increased specificity of the MRM^3 scan removed the interference peak from the matrix blank, resulting in a lower LOQ of 0.0025 µg/kg for aflatoxin B2.[11,18] Credit: SCIEX.

With stringent regulations governing toxin levels in food and feed, analytical technologies must ensure compliance with international standards set by agencies such as the European Food Safety Authority (EFSA), the US Food and Drug Administration (FDA), and the Codex Alimentarius. Developers of advanced tools like the SCIEX 7500+ system provide methods that have been optimised to align with regulatory requirements, delivering ultrafast and highly sensitive MRM acquisition for multiresidue analysis.[11,15,17]

Mass spectrometry innovations help food safety keep pace with changing environment

The impact of climate change on mycotoxin, masked mycotoxin, and tropane alkaloid contamination presents a formidable challenge to global food safety. Rising temperatures and shifting weather patterns are driving an increase in fungal proliferation and the spread of toxic plant species, necessitating advanced analytical solutions to mitigate risks. Mass spectrometry and portable detection technologies are revolutionising toxin analysis by offering high sensitivity and rapid multi-residue screening.

As the food industry adapts to the evolving threats posed by climate change, continued innovation in analytical testing will play a vital role in ensuring food safety. Laboratories equipped with cutting-edge MS systems and other advanced analytical platforms can provide accurate and reliable detection of current and emerging contaminants of concern. The integration of these technologies into routine food safety testing can help mitigate risks, futureproof against evolving regulatory changes, and protect from the growing threats posed by climate change.

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