

# Quantification of aflatoxin M<sub>1</sub> in milk using the SCIEX QTRAP 4500 LC-MS/MS system

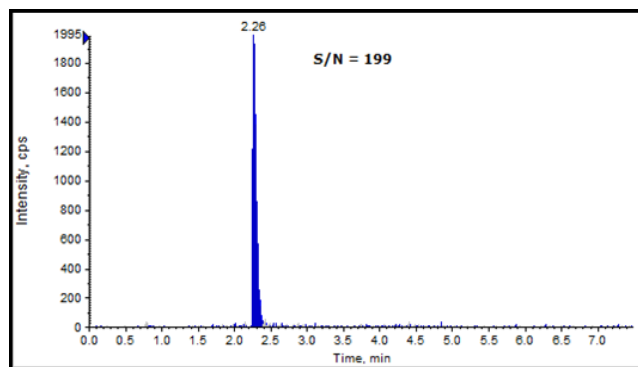
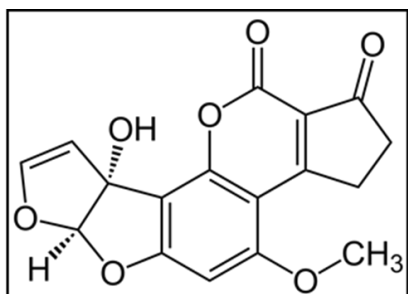
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Milk is a very healthy, nutritious food, full of key nutrients for growth. In 2018, about 66.8 million metric tons of milk were consumed in India and an average of 30 million metric tons of milk were consumed in Europe and the United states. One of the major forms of contamination that can be found in milk are mycotoxins, which are toxic compounds produced by certain fungi that can have serious health effects even at very low concentrations.<sup>1,2</sup> Regular consumption of aflatoxin M<sub>1</sub> in children may cause stunted growth, cirrhosis and cancer. Considering its serious effects in pediatrics, every government has set up stringent monitoring of aflatoxin M<sub>1</sub> in milk and its products. India as well has thus tightened the inspection of the toxins in milk samples. However, the determination of this mycotoxin in the milk samples is very difficult due to the complexity of the matrix (fat, proteins, carbohydrates, etc.).



A robust method was developed using the SCIEX QTRAP 4500 system coupled with an ExionLC system for the targeted quantification of aflatoxin M<sub>1</sub> in milk samples.



**Figure 1. Structure of aflatoxin M<sub>1</sub>.** (Top) Aflatoxin M<sub>1</sub> is a chemical compound belonging to a group of mycotoxins produced by aspergillus. C<sub>17</sub>H<sub>12</sub>O<sub>7</sub>, MW: 328.276 g/mol. (Bottom) Chromatogram showing S/N ratio of aflatoxin M<sub>1</sub> at MRL level (0.50 ppb) concentration.

## Key features of targeted quantification method for aflatoxin M<sub>1</sub>

- A targeted quantitative method has been developed on the SCIEX QTRAP 4500 system using 2 MRM transitions
- A simple sample preparation method was developed for extraction of milk samples
- Sensitivity was easily sufficient to meet the MRL requirements (0.5 ppb) for aflatoxin M<sub>1</sub> in milk
- The developed method was validated as per the regulatory guidelines described in the Commission Decision (2002/657/EC) directive<sup>3</sup>

## Methods

**Sample preparation:** Standard aflatoxin M<sub>1</sub> was purchased from Sigma Aldrich. All other chemicals used were commercially available and of LC-MS grade. Milk samples were purchased from local vendors and were stored in the refrigerator at 2 to 8 °C until sample analysis.

An optimized extraction procedure was developed in which 5 mL of milk was mixed with 10 mL of acetonitrile and vortexed for 10 min. Then, 2 g of NaCl was added, vortexed for 5 min and centrifuged at 3200 rpm for 5 min at 4 °C. The organic layer was collected and evaporated to dryness under nitrogen. The residue was reconstituted with 1 mL with 90:10 water/methanol, filtered and transferred into autosampler vials for LC-MS/MS analysis.

**Chromatography:** Separation was performed using an ExionLC system with a Kinetex C18 column (1.7 µm, 100 Å, 150 x 2.1 mm). An injection volume of 20 µL was used. The gradient program is shown in Table 1.

**Table 1. Gradient profile and mobile phase composition.**

Total Time (min)	Flow Rate (µL/min)	A%	B%
0.00	500	90	10
4.00	500	5	95
4.10	500	90	10
7.50	500	90	10

Mobile phase A: water + 0.1% formic acid

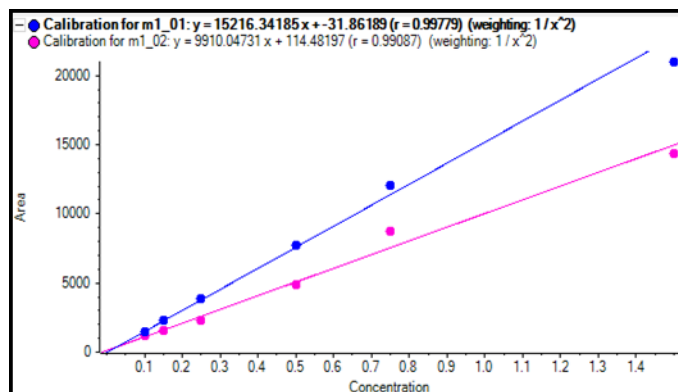
Mobile phase B: acetonitrile + 0.1% formic acid

**Mass spectrometry:** The SCIEX QTRAP 4500 system was operated in multiple reaction monitoring (MRM) mode. The Turbo V ion source was used with an electrospray ionization (ESI) probe, in positive polarity at an ion spray voltage of 2800. The MRM transitions used are shown in Table 2. Analyst software 1.7 was used for method development and data acquisition.

**Data processing:** LC-MS/MS data was processed using the MultiQuant software 3.0.3.

**Table 2. MRM transitions of aflatoxin M<sub>1</sub>.**

Compound	Precursor Ion	Product Ion (Quantifier)	Product Ion (Qualifier)	Collision Energy (CE)
Aflatoxin M <sub>1</sub>	329.1	259.1	273.0	51 / 44

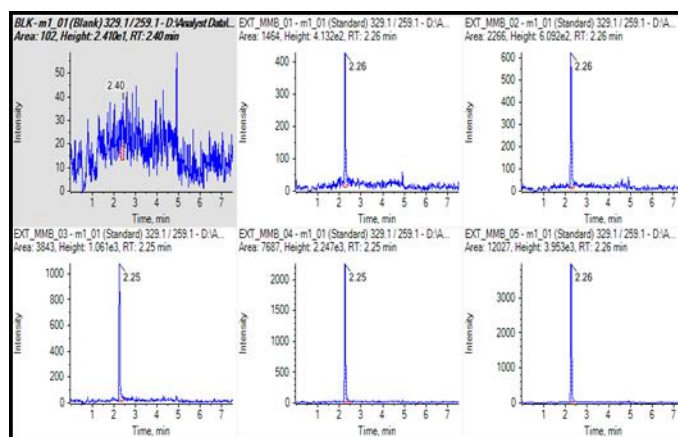


Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
m1_01	0.15	10 of 10	2.630e3	6.692e1	2.54

**Figure 2. Calibration curve of aflatoxin M<sub>1</sub> in milk.** Good linearity was achieved for the concentration range evaluated (0.15 to 1.50 ppb) with *r* values >0.99 for both MRM transitions, with 1/*x*<sup>2</sup> weighting. Good reproducibility was observed for aflatoxin M<sub>1</sub> in extracted samples at the LOD level of 0.15ppb, 2.54 %CV for 6 replicates.

## Results

First, the chromatography was optimized to provide a fast 7.5 min run time (Figure 1). Calibration curves were generated across a concentration range of 0.15 to 1.50 ppb of aflatoxin M<sub>1</sub> in extracted milk matrix to determine the performance of the method. A LOD of 0.15 ppb was achieved in extracted milk and the %CV (*n*=10) was still <5%. Good linearity across the range was observed (Figure 2). Reproducibility was evaluated between and within days with the 6 replicates injections and the %CV was found to be <5%, which proves the method is robust and reproducible below its MRL level (0.50 ppb) (Figure 3, bottom).



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
m1_01	0.50	6 of 6	9.182e3	3.671e2	4.00

**Figure 3. Representative chromatograms of aflatoxin M<sub>1</sub>.** The integrated peaks for aflatoxin M<sub>1</sub> from 0.15 ppb to 1.5 ppb are shown here, including the blank.

No significant matrix interferences were observed (Figure 3, top). Ion ratios were used to ensure that the correct peaks were integrated in each sample, for added confidence in detection (Figure 4).

## Conclusions

The method developed here enables the fast and accurate quantification and confirmation of aflatoxin M<sub>1</sub> in extracted milk samples by LC-MS/MS. The SCIEX QTRAP 4500 system provides good sensitivity and selectivity for this analysis, with minimal sample preparation, providing maximized throughput for the analysis of large sample batches in a short time period. The method was developed in accordance with the Commission Decision (2002/657/EC) directive recommendations and showed acceptable accuracies (70%-130%) across the calibration curves in matrix, had good linearity for both the MRM transitions and had high repeatability with %CV < 5%.

This rapid and sensitive LC-MS/MS method for the quantification of aflatoxin M<sub>1</sub> in milk also easily meets the MRL of 0.5 ppb level for this toxin in milk.

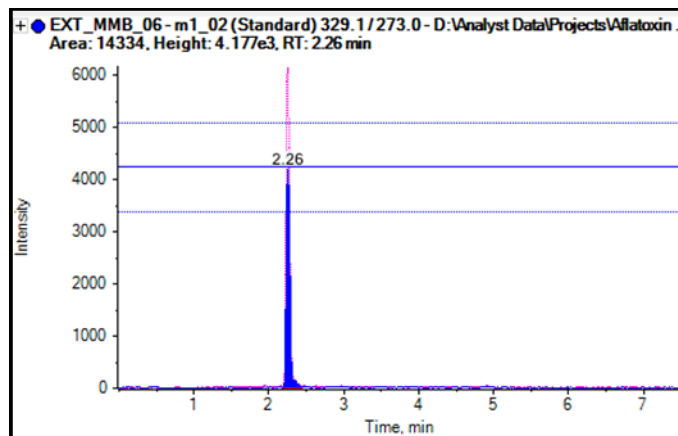


Figure 4. Representation of the aflatoxin M<sub>1</sub>, with 20% difference in the ion ratio.

## References

1. Zhang K *et al.* (2017) Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). [\*J. Agric. Food Chem.\* \*\*65\*\*\(33\), 7138-7152.](#)
2. [Carcinogen Aflatoxin detected in FSSAI milk survey samples.](#) (2019)
3. [EC European Commission, Commission Decision 2002/657/EC of 12 August 2002.](#) Off. J Eur. Communities, L221, 8–36.

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