Quantitative multiple reaction monitoring (MRM) of toxic metabolite aflatoxin-M1 from milk samples with atmospheric pressure matrix-assisted laser desorption/ionization (AP/MALDI)

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INTRODUCTION

Aflatoxin M1 (AFM1) is a carcinogenic, hydroxylated metabolite of aflatoxin B1 (AFB1), which is commonly found in milk. It is relatively stable to decontamination procedures. Its presence in milk and dairy products poses a major risk to human health mandating its maximum limits. AP/MALDI is a versatile and high throughput ionization source that can be interchanged with an ESI source of most OEM mass analyzers. In this work, we demonstrate rapid, chromatography-free and quantitative AP/MALDI multiple reaction monitoring (MRM) analysis of AFM1 from milk samples.

MATERIALS AND METHODS

Sample Preparation:

50 mL of the milk sample was homogenized at 4000 rpm for 5 min, heated and centrifuged at 7000 rpm for 5 min to separate the milk solids. The upper lipid layer was discarded. 30 mL of the remaining milk sample was aliquoted into three 50 mL centrifuge tubes, with 10 mL in each tube. To these tubes, 10 mL of deionized water was added and vortexed for 1 min. This sample was further processed for the extraction of AFM1 via immunoaffinity chromatography (IAC method). The recovery percentage of AFM1 obtained with QTRAP 5500 was analysed by spiking the residue-free milk samples with AFM1 at 0.25 ng/mL and 0.5 ng/mL concentration. These were subsequently diluted with deionized water and subjected to IAC clean-up. Samples were premixed with CHCA matrix and spotted on a 192-well target plate.

MassTech AP/MALDI system

AP/MALDI (ng) UHR source (MassTech, Inc., Columbia, MD) with a 355 nm Nd: YAG laser source was used for the experiment. Laser energy used for desorption of samples was 80%. The laser firing pattern used was constant speed raster motion. Optimization of parameters such as laser energy, de-clustering potential, collision energy and laser energy was done using reference standard solutions.

SCIEX Qtrap 5500 system

The QTRAP 5500 mass spectrometer coupled with AP/MALDI was used to acquire MRM ion spectra at IonSpray voltage floating 4000 V, curtain gas as 10 and interface heater temperature (220°C), De-clustering Potential (DP) as 91 V and collision energy (CE) 33 eV for 273.040u and 45 eV for 229.007u to achieve fragmentation.

Ultra-High Performance Liquid Chromatography with Fluorescence Detection (UHPLC-FLD)

The validation was performed using UHPLC FLD on an Acquity H-Class UHPLC-FLD (Waters Corporation, Manchester, UK) instrument. The column temperature was maintained at 40 ºC and the flow rate as 0.4 mL/min with injection volume of 10 µL. The excitation wavelength was 365 nm and the emission wavelength was set at 456 nm. For data processing and analysis, Empower_3 software (Waters) was used.

Figure 1. AP/MALDI coupled with SCIEX QTRAP 5500 system

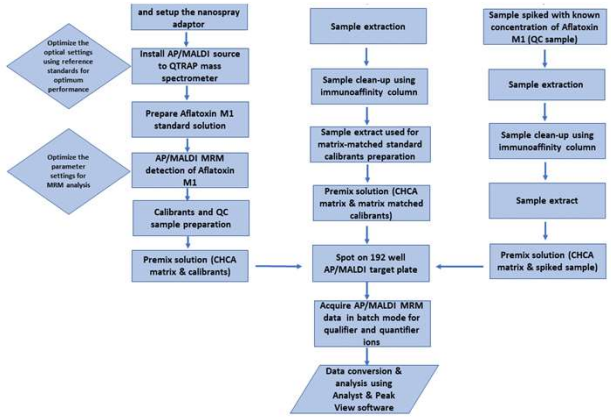

Figure 2. Process flow analysis of Aflatoxin M1 AP/MALDI SCIEX Qtrap 5500

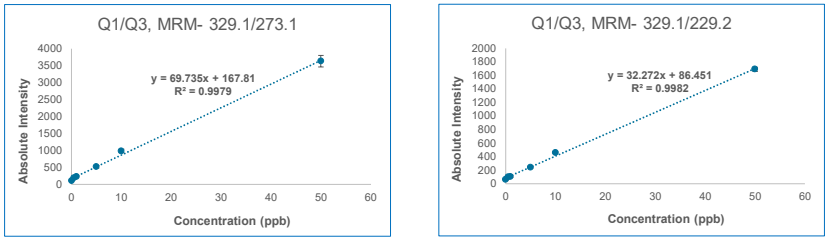

Figure 3. Solvent standard calibration model for aflatoxin M1 Q1/Q3, MRM- 329.1/273.1 & Q1/Q3, MRM- 329.1/229.2 across a concentration range of 0.02-50 ppb.

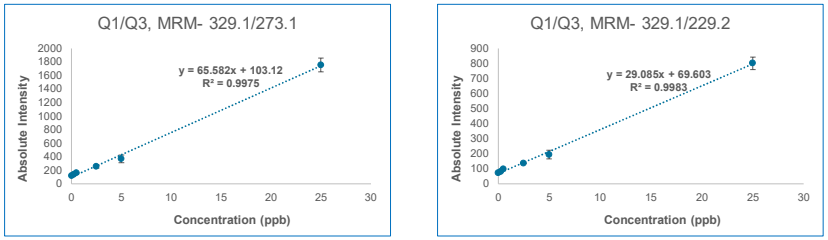

Figure 4. Matrix-matched calibration model for aflatoxin M1 Q1/Q3, MRM- 329.1/273.1 & Q1/Q3, MRM- 329.1/229.2 across a concentration range of 0.01-25 ppb

Conc. (ppb)	Ion ratio (quantifier: qualifier) (n=3)			Avg.	SD	RSD (%)
25	221.2	219.7	216.4	219.1	2.4	1.1
5	188.8	189.0	192.4	190.1`	2.0	1.1
2.5	184.3	200.9	174.5	186.6	13.3	7.1
0.5	175.3	161.1	157.1	164.5	9.6	5.8
0.25	169.1	179.9	161.2	170.1	9.4	5.5
0.01	160.8	161.9	169.4	164.0	4.7	2.9
Two fold diluted QC samples and milk control						
Control	164.4	157.2	164.8	162.1	4.3	2.6
0.25*	157.5	171.0	183.3	170.6	12.9	7.6
0.5*	165.3	156.6	165.0	162.3	4.9	3.1

Table 1.. Ion ratios (IR) of qualifier (273.1u) to quantifier (229.2u) for AFM1 from matrix-matched calibrants, quality controls and milk control samples

*Note: The amounts spiked in milk were 0.2 and 0.4 ppb. After diluting the milk matrix, concentrating during clean-up procedure and premixing with MALDI matrix, the amounts correlate to 0.25 and 0.5 ppb.

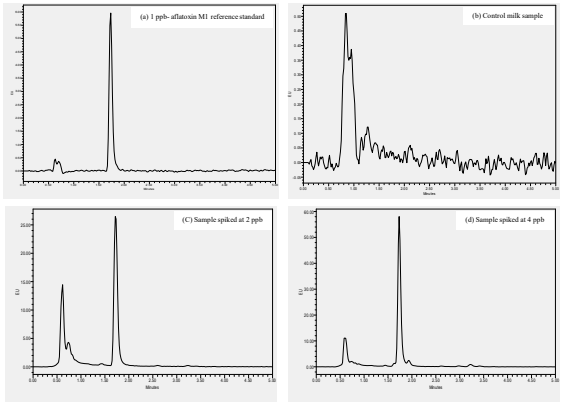

Figure 5. UHPLC-FLD chromatograms of aflatoxin M1 reference standard at concentrations of (a) 1 ppb, (b) control milk sample and sample spiked at (c) 2 ppb and (d) 4 ppb.

RESULTS

Two MRM transitions of AFM1, namely, Q1/Q3; 329.1/273.1 and Q1/Q3; 329.1/229.2 were observed and monitored from the standard and spiked milk samples. A limit of detection (LOD) of 0.01 µg/kg was achieved from the calibrants of AFM1 extracted in milk matrix and reference standards using both the MRM transitions. The observed LOD matches with the established maximum limits (ML) of Codex Alimentarius collection of food standards, FDA, USA (0.5 µg/kg) and EU (0.05 µg/kg). Peak intensities of the individual MRM transitions were plotted against the reference and spiked concentrations. Excellent linearity with regression coefficients (R2) were observed (0.99) for both the MRM transitions with standards and matrix-matched calibrants across the calibration ranges 0.02 to 50 µg/kg (reference standard calibrants) and 0.01 to 25 µg/kg (matrix-matched calibrants). Recoveries of AFM1 from two different QC samples (0.25 and 0.5 µg/kg) were estimated with the calibration curve of MRM transitions from matrix-matched calibrants with replicates (n=3). MRM transition Q1/Q3; 329.1/229.2 exhibited better recovery yields for the two QC concentrations (106 and 97.4% for 0.25 and 0.5 µg/kg) than MRM transition Q1/Q3; 329.1/273.1 (100.2% for 0.5 µg/kg).

METHOD HIGHLIGHT

- A chromatography-free method for detection of aflatoxin M1 from milk samples was developed.
- AP/MALDI MRM quantitation showed excellent accuracy and repeatability. Ion ratios increase the confidence of the measurement.
- The quantitative parameters of AP/MALDI method were in agreement with the accepted 'gold-standard' method of UHPLC FLD analysis.
- High throughput AP MALDI source coupled with the high sensitivity of SCIEX QTRAP 5500 offers a robust platform for the analysis of aflatoxin M1 from milk and other food matrices.

REFERENCES

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