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Assessment of the Levels of Aflatoxins in Silver Cyprinid Sold at Kansanga-Kampala Uganda

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ABSTRACT

Aflatoxins, toxic secondary metabolites produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*, are a significant concern for food safety due to their carcinogenic and immunosuppressive effects. They frequently contaminate staple foods, including fish products, in warm, humid environments. Silver cyprinid, commonly known as silverfish, is an essential and affordable source of protein in Uganda, consumed by many households. However, the drying, storage, and handling practices involved in its sale raise concerns about the potential for aflatoxin contamination, particularly in local markets. This study aimed to assess the levels of aflatoxins in silver cyprinid sold in Kansanga-Kampala. A total of 10 silver cyprinid samples were collected from different vendors in Kansanga for analysis. The study employed Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), a highly sensitive and specific method, to detect and quantify the presence of aflatoxins in these samples. The analysis involved the preparation of aflatoxin standard solutions (B1, B2, G1, and G2) to create calibration curves by plotting response against concentration. Calibration curves were linear, with coefficients of determination (R^2 values) greater than 0.99 for all aflatoxin types. An injection volume of 1µL was used and 7 levels of the aflatoxin standards were employed having 1,5,10,25,50,75 and 100ppb. However, results from the analysis of silverfish samples showed no detectable levels of aflatoxins (ND), suggesting that the silverfish from this particular market were free from aflatoxin contamination. This is a positive finding for public health as aflatoxins are highly carcinogenic and can lead to liver cancer, immune suppression, and stunted growth in children when consumed over time. It indicates that silverfish sold in Kansanga, Kampala, during the sampling period may be considered safe for consumption with respect to aflatoxin contamination. While this result is positive, the potential for aflatoxin contamination remains a concern due to environmental factors such as humidity and temperature, which can fluctuate throughout the year. Therefore, regular monitoring of aflatoxin levels in silver cyprinid and other food products is recommended to ensure ongoing food safety. In conclusion, this study establishes a baseline for aflatoxin contamination in silver cyprinid sold in Kansanga and demonstrates that, during the period of analysis, the risk of aflatoxin exposure from silver cyprinid consumption is negligible. However, to maintain this standard, it is crucial to implement sustained preventive measures, including better storage infrastructure and vendor education to safeguard public health and the integrity of the local food supply.

Keyword: Aflatoxins; Silver cyprinid; Food safety; LC-MS/MS; Public health

INTRODUCTION

Silver cyprinid (*Rastrineobola argentea*), a small pelagic fish species, locally known as mukene in Uganda [1] is a vital source of protein and essential nutrients for many communities in Uganda, particularly in urban areas like Kansanga, Kampala. Its affordability and high nutritional content make it a popular food choice among various socioeconomic groups [2]. Silver cyprinid is not only consumed directly but is also used in the preparation of other food products, such as fish powder, which is added to meals to enhance nutritional value. However, despite its benefits, silver cyprinid is susceptible to contamination by aflatoxins a group of toxic metabolites produced by molds, particularly Aspergillus flavus and *Aspergillus parasiticus*. Aflatoxins are known for their carcinogenic properties and are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) [3]. The consumption of aflatoxin-contaminated food can lead to severe health consequences, including liver cancer, immune suppression, and stunted growth in children [4]. Chronic exposure to aflatoxins, even at low levels, poses a significant public health risk, especially in regions where food safety controls may be inadequate. In Uganda, aflatoxin contamination is primarily associated with staple crops such as maize and groundnuts [5, 6]. However, recent studies have indicated that fish, including silver cyprinid, can also be contaminated with aflatoxins, particularly when exposed to poor post-harvest handling and storage practices [7]. Silver cyprinid

is often dried in open environments [8], where they are vulnerable to mold growth, especially under humid conditions. The presence of aflatoxins in silver cyprinid is a growing concern [9], as it directly impacts the safety and quality of this widely consumed food source. Despite the potential health risks, there is limited research on the levels of aflatoxins in silver cyprinid sold in Ugandan markets, including Kansanga. The lack of data on aflatoxin contamination in silver cyprinid highlights a critical gap in food safety knowledge, which this study aims to address.[10, 11] By assessing the levels of aflatoxins in silver cyprinid sold at Kansanga, Kampala, this research seeks to provide essential data that can inform public health policies and interventions. Understanding the extent of aflatoxin contamination in this popular food source is crucial for protecting consumer health, ensuring food safety, and guiding future regulatory measures in Uganda [12]

Silver cyprinid (Rastrineobola argentea) is an essential dietary staple in Uganda, widely consumed for its high protein content, affordability, and ease of availability, particularly in urban centers such as Kansanga. As a key source of protein, silver cyprinid plays a vital role in food security, especially among low-income households. However, despite its nutritional importance, silver cyprinid is increasingly at risk of contamination by aflatoxins, a group of potent toxins produced by certain species of molds, particularly Aspergillus flavus and Aspergillus parasiticus. Aflatoxins are among the most dangerous mycotoxins known, with the potential to cause severe health problems, including acute poisoning, liver damage, and cancer[13, 14]. While aflatoxin contamination has been well-documented in staple crops such as maize and groundnuts, the risk of aflatoxins in fish products, including silver cyprinid, has received less attention[15, 16]. This is particularly concerning given that fish, during drying and storage can become contaminated by mold spores, especially in environments with poor hygiene and inadequate storage practices, conditions that are unfortunately common in many markets across Uganda. Silver cyprinid sold in open markets are often dried in unsanitary conditions and stored in ways that expose them to moisture and mold contamination, increasing the likelihood of aflatoxin presence[17, 18]. Despite these risks, there is a significant gap in the available data regarding the levels of aflatoxins in silver cyprinid sold at Kansanga. This lack of information poses a critical public health challenge, as consumers are at risk of unknowingly ingesting harmful levels of aflatoxins, leading to long-term health consequences. Without thorough testing and quality control measures, contaminated fish can easily enter the food supply chain, reaching consumers who are unaware of the potential dangers[19, 20]. Therefore, this study aims to assess the levels of aflatoxins in silver cyprinid sold at Kansanga-Kampala, Uganda. By conducting a thorough investigation into the concentration of aflatoxins in this critical food source, the research will fill a vital gap in the current knowledge, providing evidence that could lead to improved food safety regulations, better handling and storage practices, and increased public awareness. Addressing the issue of aflatoxin contamination in silver cyprinid is not only crucial for safeguarding public health but also for ensuring the continued availability of safe, nutritious food in Uganda's markets.

The study on assessing aflatoxin levels in silver cyprinid sold at Kansanga market in Kampala, Uganda, is of critical importance due to its potential impact on public health, food safety, and economic stability. Aflatoxins, known carcinogens, pose severe health risks, including liver cancer and immune suppression, making it essential to evaluate their presence in silver cyprinid, a major source of protein for many Ugandans. By providing data on aflatoxin contamination, this research will inform public health interventions and help develop improved food safety practices, ensuring that the silver cyprinid consumed by the population is safe. Additionally, the findings could influence policymakers and regulatory bodies to strengthen food safety standards and monitoring processes, contributing to a more robust food safety framework in Uganda. The study also holds economic significance, as ensuring the safety of silver cyprinid can maintain consumer trust and support the livelihoods of those involved in its trade. The research will also add to the academic knowledge on aflatoxins in other food items and raise awareness among consumers and vendors about the importance of proper handling and storage practices. In doing so, it will promote behavioral changes that reduce the risk of contamination, ultimately improving public health and fostering a safer food environment in Kampala's markets.

The study on assessing aflatoxin levels in silver cyprinid, is crucial due to the potential public health risks posed by aflatoxin contamination in a widely consumed food source. Silver cyprinid is a staple protein for many Ugandans, particularly in urban areas, and any contamination could have severe implications, including liver cancer, immune suppression, and growth impairment, especially in vulnerable populations such as children. Despite its importance in the diet, there is a significant lack of data on aflatoxin contamination in silver cyprinid, with most research focusing on crops like maize and groundnuts. This gap in knowledge is alarming, given the potential for contamination during the drying, storage, and handling processes used in Kansanga. Since this study offers the first thorough evaluation of aflatoxin levels in silver cyprinid in this market, it is justified by its ability to close this important information gap. In order to lower aflatoxin exposure, market behaviors, regulations,

and public health initiatives will all be greatly influenced by the findings. Furthermore, the research will increase the understanding of the significance of appropriate food handling and storage procedures for preventing contamination among suppliers, buyers, and legislators. In order to preserve public health and guarantee the continued safety and dependability of this vital food supply, the study will determine the factors influencing aflatoxin presence in silver cyprinid and provide practical recommendations to improve food safety. Additionally, the results may provide a foundation for other studies on aflatoxin contamination in other food types, contributing to a broader understanding of food safety in Uganda.

METHODOLOGY

Materials, reagents and equipment

Polyethylene bags, Permanent marker, Analytical balance (Mettler Toledo), Centrifuge, Pre-sterilized centrifuge tubes from GenFollower, Solid-Phase Extraction (SPE) Cartridges, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) System, Disposable gloves (Nitrile Powder free examination Glove-Medium size), Digital shaker, Reagent dispenser, Vacuum manifold, Methanol, formic acid, vacuum pump, distilled water, Phosphate Buffered Saline (containing 8.0094g of NaCl, 0.2026g of KCl, 1.4092g of Na_2HPO_4 and 0.2450g of KH_2PO_4), Analytical standards of aflatoxins (Aflatoxin B1, B2, G1 and G2), sample vials, glass bottles.

Sampling

Random sampling technique was employed to ensure that silver cyprinid samples are collected randomly from different vendors within Kansanga to ensure diversity and representativeness. Silver cyprinid samples of approximately equal amount were collected directly from the different vendors and were placed in clean polyethylene bags to prevent contamination and moisture ingress. Each sample was labeled with a unique identifier that is A, B, C, D, E, F, G, H, I, J according to the point from where it had been collected. The sampling area was Kansanga which is a densely-populated area and an active urban center. 4 samples were collected from different vendors (one sample from each) around Wonder World Amusement Park and UK Mall and these samples were labeled B, E, F and J. Samples A, D and C were collected from 3 different vendors who were very distant from each other in Nabutiti. The other 3 samples were collected from; one vendor in Kiyembe Market which was labeled sample I and another vendor who was selling from a Kiosk almost near Bank of Baroda which was sample G. The last sample was collected from another vendor near UBA Bank which was labeled sample H. The samples were transported immediately to the laboratory to preserve their condition and prevent any further fungal growth that might alter aflatoxin levels.

Figure 1: Extracted Google map showing the location of Kansanga (Source: Google map.com)

Sample Preparation

5.0000g of the samples were weighed using an analytical balance into 50ml centrifuge tubes which were well labeled respectively to the sample and then racked.

Aflatoxin Extraction

The extraction of aflatoxins from the silver cyprinid samples followed a standardized solvent extraction protocol suitable for subsequent LC-MS/MS analysis:

A mixture of methanol and water of ratio (60:40 v/v) was used as the extraction solvent. 10ml of the solvent was added to the samples in the centrifuge tubes using a reagent dispenser. The tubes containing the samples were then placed on a digital shaker at a speed of 200 rpm for 30 minutes to enable extraction of aflatoxins from the silver cyprinid into the solvent mixture that was added. The mixture was centrifuged at 3500 rpm for 5 minutes to separate the solid residue from the liquid extract using a centrifuge which was then put into bottles.

The residue was rinsed with 10mls of PBS (Phosphate Buffered Saline) after removing the liquid extract. The mixture was centrifuged again at 3500 rpm for 5 minutes for further extraction of aflatoxins from the residue. The liquid extract obtained was added to the bottles containing the first liquid extract. Since the liquid extract obtained was turbid, it was centrifuged again at 3500 rpm for 5 minutes to obtain a clearer solution. This was done in smaller centrifuge tubes. The centrifuged solution (liquid extract) was transferred back into the bottles.

Cleanup (Solid-Phase Extraction)

The aflatoxins were purified using solid-phase extraction cartridges having Aflasta Immuno Affinity Column (AIAC). Only aflatoxins are attached to the AIAC and the rest co-extractants pass through. The apparatus used is called vacuum manifold. The vacuum manifold was connected to a vacuum pump which creates a negative pressure in the bottles containing the liquid extract, causing a suction pressure which leads to the sucking of the liquid.

Empty bottles in which the liquid had been fully sucked up were rinsed with 10mls of PBS and it was also allowed to be fully sucked up. Aflatoxins obtained were eluted from the SPE cartridges using 1.0ml of methanol into sample vials which were well labeled respectively according to the sample, and purified extract ready for LC-MS/MS analysis was obtained. The methanol breaks the bond between the bound aflatoxin and the material in the Aflasta column. It has a higher affinity for aflatoxins more than the sorbent material.

Quantitative Analysis Using LC-MS/MS

The purified aflatoxin extracts were analyzed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), a highly sensitive and specific method for detecting and quantifying aflatoxins. The LC-MS/MS system was equipped with a reverse-phase C18 column and an electrospray ionization (ESI) source operating in positive ion mode. This setup is optimized for aflatoxin detection. Two mobile phases which included mobile phase A and B were used. Mobile phase A was 5 millimolar Ammonium Formate + 0.1% formic acid in water and mobile phase B was 5 millimolar Ammonium Formate + 0.1% formic acid in methanol. Aflatoxins B1, B2, G1, and G2 were not detected in the samples. Calibration curves were generated using aflatoxin standards to quantify the levels in the samples.

The sample vials, containing the aflatoxin extract dissolved in the mobile phase, were loaded into the LC auto-sampler. This instrument automates the injection of precise sample volumes. An injection volume of 1.0 microliter was used. After injection, the sample passed through a chromatography column filled with a stationary phase that interacted with the aflatoxins differently based on their chemical properties. Aflatoxins (B1, B2, G1, and G2) have varying affinities for the column's stationary phase, meaning they travel through the column at different rates, thus getting separated from each other. The mobile phase helps carry the sample through the column. The flow rate and solvent gradient were controlled to optimize separation and to ensure accuracy.

The aflatoxins were eluted from the chromatography column one by one based on their retention times (how long they take to travel through the column). Each aflatoxin type (B1, B2, G1, and G2) had a specific retention time, which was established using aflatoxin standards during calibration. After the chromatographic separation, the aflatoxins entered the mass spectrometry (MS/MS) system for detection and quantification. They must be ionized to be detected by the mass spectrometer.

The most common ionization techniques used in LC-MS/MS are Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). In Electrospray Ionization, the liquid sample leaving the chromatography column is sprayed through a fine nozzle, creating a mist of droplets. A high voltage is applied to the nozzle, charging the droplets. As the solvent evaporates, the molecules in the droplets become charged ions. ESI is especially useful for polar large compounds like aflatoxins. In Atmospheric Pressure Chemical Ionization (APCI), the sample is nebulized into a gas and passed through a high-voltage electric field to ionize it. APCI is often used for less polar, more volatile compounds. For aflatoxin analysis, positive ionization is typically used because it offers better sensitivity for the molecules.

After ionization, the aflatoxins were sent into the first mass analyzer (MS1), which separated the ions based on their mass-to-charge ratio (m/z). The mass analyzer in MS1 selected a particular ion called the parent ion from the sample, based on its m/z. Each type of aflatoxin has a unique m/z value

that allows it to be identified and selected for further analysis. The parent ion was then fragmented into smaller pieces called the daughter ions or fragment ions. They were then passed to the second mass analyzer for further analysis for the confirmation of identity and quantification of each type of aflatoxin. The aflatoxins were quantified based on the intensity of the detected ions. In LC-MS/MS, the intensity of the peaks (representing ion counts) in the chromatogram is proportional to the concentration of the aflatoxins. The sensitivity of LC-MS/MS allows for the detection of aflatoxins at very low levels ensuring that even trace contamination can be identified.

Quality Control

The LC-MS/MS system was calibrated with aflatoxin standards before analysis to ensure accurate quantification. Each sample was analyzed in duplicate to minimize analytical errors and ensure reproducibility. Blank samples (samples without aflatoxins) were run periodically to ensure there is no contamination or carry-over from previous samples.

RESULTS

Table 1: Tabular results for weight of samples used

Sample	Weight (g)
A	5.0669
B	5.0037
C	5.0675
D	5.0138
E	5.0385
F	5.0089
G	5.0356
H	5.0245
I	5.0339
J	5.0392

Table 2: Quantitation Results for Aflatoxin G2

Data File	Compound	Sample Type	RT	Resp.	Final Conc	Recovery
Blank.d	Aflatoxin G2	Blank	4.645	7	ND	
Aflatoxin STD 1ppb.d	Aflatoxin G2	Calibration	4.427	487	ND	0.00
Aflatoxin STD 5ppb.d	Aflatoxin G2	Calibration	4.482	2093	4.5963	91.93
Aflatoxin STD 10ppb.d	Aflatoxin G2	Calibration	4.482	3104	10.6440	106.44
Aflatoxin STD 25ppb.d	Aflatoxin G2	Calibration	4.482	5505	25.0078	100.03
Aflatoxin STD 50ppb.d	Aflatoxin G2	Calibration	4.482	9649	49.8067	99.61
Aflatoxin STD 75ppb.d	Aflatoxin G2	Calibration	4.509	13790	74.5787	99.44
Aflatoxin STD 100ppb.d	Aflatoxin G2	Calibration	4.509	18100	100.3665	100.37
PRL 120-24 A-r001.d	Aflatoxin G2	Sample	4.183	7	ND	
PRL 120-24 A-r002.d	Aflatoxin G2	Sample	4.726	18	ND	
PRL 120-24B -r001.d	Aflatoxin G2	Sample	4.563	9	ND	
PRL 120-24B -r002.d	Aflatoxin G2	Sample	4.427	5	ND	
PRL 120-24 C-r001.d	Aflatoxin G2	Sample	3.938	5	ND	
PRL 120-24 C-r002.d	Aflatoxin G2	Sample	4.645	4	ND	
PRL 120-24 D-r001.d	Aflatoxin G2	Sample	4.835	2	ND	
PRL 120-24 D-r002.d	Aflatoxin G2	Sample	4.590	2	ND	
PRL 120-24 E-r001.d	Aflatoxin G2	Sample	4.563	1	ND	
PRL 120-24 E-r002.d	Aflatoxin G2	Sample	4.536	4	ND	
PRL 120-24 F-r001.d	Aflatoxin G2	Sample	4.590	19	ND	
PRL 120-24 F-r002.d	Aflatoxin G2	Sample	4.699	4	ND	
PRL 120-24 G-r001.d	Aflatoxin G2	Sample	4.672	13	ND	
PRL 120-24 G-r002.d	Aflatoxin G2	Sample	4.482	3	ND	
PRL 120-24 H-r001.d	Aflatoxin G2	Sample	4.672	1	ND	
PRL 120-24 H-r002.d	Aflatoxin G2	Sample	4.074	37	ND	
PRL 120-24 I-r001.d	Aflatoxin G2	Sample	3.748	32	ND	
PRL 120-24 I-r002.d	Aflatoxin G2	Sample	4.889	4	ND	
PRL 120-24J-r001.d	Aflatoxin G2	Sample	4.726	7	ND	
PRL 120-24J-r002.d	Aflatoxin G2	Sample	4.780	6	ND	

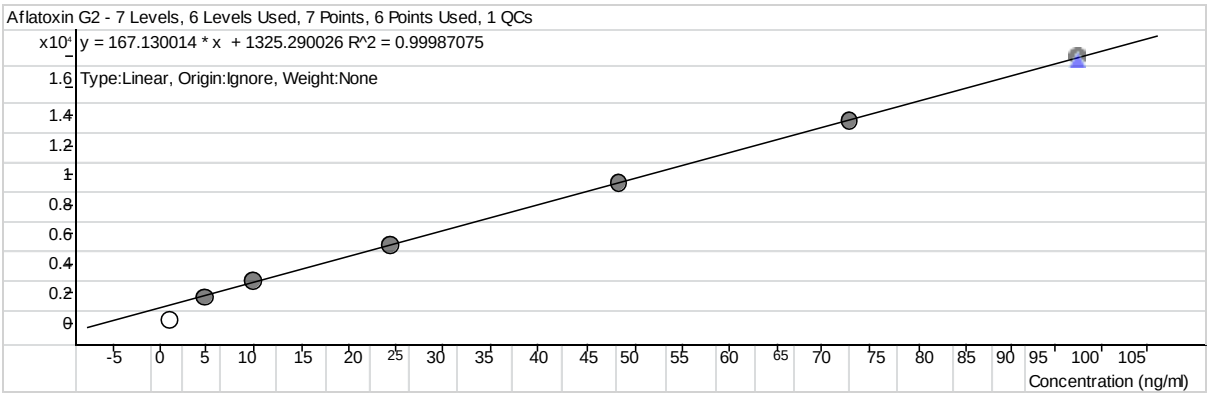

Figure 2: A Calibration curve for Aflatoxin G2 showing Response against Concentration

Table 3: Quantitation Results for Aflatoxin G1

Data File	Compound	Sample Type	RT	Resp.	Final Conc.	Recovery
Blank.d	Aflatoxin G1	Blank	4.624	3	ND	
Aflatoxin STD 1ppb.d	Aflatoxin G1	Calibration	4.651	1079	ND	0.00
Aflatoxin STD 5ppb.d	Aflatoxin G1	Calibration	4.706	5110	4.4808	89.62
Aflatoxin STD 10ppb.d	Aflatoxin G1	Calibration	4.706	7920	9.8435	98.44
Aflatoxin STD 25ppb.d	Aflatoxin G1	Calibration	4.706	16001	25.2627	101.05
Aflatoxin STD 50ppb.d	Aflatoxin G1	Calibration	4.733	30010	51.9935	103.99
Aflatoxin STD 75ppb.d	Aflatoxin G1	Calibration	4.733	40894	72.7613	97.02
Aflatoxin STD 100ppb.d	Aflatoxin G1	Calibration	4.733	55514	100.6582	100.66
PRL 120-24 A-r001.d	Aflatoxin G1	Sample	4.787	19	ND	
PRL 120-24 A-r002.d	Aflatoxin G1	Sample	4.706	12	ND	
PRL 120-24B -r001.d	Aflatoxin G1	Sample	5.059	4	ND	
PRL 120-24B -r002.d	Aflatoxin G1	Sample	5.032	12	ND	
PRL 120-24 C-r001.d	Aflatoxin G1	Sample	4.651	2	ND	
PRL 120-24 C-r002.d	Aflatoxin G1	Sample	4.896	3	ND	
PRL 120-24 D-r001.d	Aflatoxin G1	Sample	4.488	11	ND	
PRL 120-24 D-r002.d	Aflatoxin G1	Sample	5.086	5	ND	
PRL 120-24 E-r001.d	Aflatoxin G1	Sample	4.624	19	ND	
PRL 120-24 E-r002.d	Aflatoxin G1	Sample	4.679	9	ND	
PRL 120-24 F-r001.d	Aflatoxin G1	Sample	4.787	4	ND	
PRL 120-24 F-r002.d	Aflatoxin G1	Sample	4.896	4	ND	
PRL 120-24 G-r001.d	Aflatoxin G1	Sample	4.380	44	ND	
PRL 120-24 G-r002.d	Aflatoxin G1	Sample	4.624	13	ND	
PRL 120-24 H-r001.d	Aflatoxin G1	Sample	5.575	9	ND	
PRL 120-24 H-r002.d	Aflatoxin G1	Sample	4.488	15	ND	
PRL 120-24 I-r001.d	Aflatoxin G1	Sample	4.814	18	ND	
PRL 120-24 I-r002.d	Aflatoxin G1	Sample	4.760	11	ND	
PRL 120-24J-r001.d	Aflatoxin G1	Sample	4.434	8	ND	
PRL 120-24J-r002.d	Aflatoxin G1	Sample	4.516	8	ND	

Table 4: Quantitation Results for Aflatoxin B2

Data File	Compound	Sample Type	RT	Resp.	Final Conc.	Recovery
Blank.d	Aflatoxin B2	Blank	5.609	2	ND	
Aflatoxin STD 1ppb.d	Aflatoxin B2	Calibration	5.174	587	ND	0.00
Aflatoxin STD 5ppb.d	Aflatoxin B2	Calibration	5.147	3525	4.6562	93.12
Aflatoxin STD 10ppb.d	Aflatoxin B2	Calibration	5.229	6112	10.9617	109.62
Aflatoxin STD 25ppb.d	Aflatoxin B2	Calibration	5.229	12236	25.8907	103.56
Aflatoxin STD 50ppb.d	Aflatoxin B2	Calibration	5.229	21316	48.0258	96.05
Aflatoxin STD 75ppb.d	Aflatoxin B2	Calibration	5.229	32020	74.1205	98.83
Aflatoxin STD 100ppb.d	Aflatoxin B2	Calibration	5.229	43188	101.3451	101.35
PRL 120-24 A-r001.d	Aflatoxin B2	Sample	5.337	4	ND	
PRL 120-24 A-r002.d	Aflatoxin B2	Sample	4.957	1	ND	
PRL 120-24B -r001.d	Aflatoxin B2	Sample	4.740	1	ND	
PRL 120-24B -r002.d	Aflatoxin B2	Sample	5.174	1	ND	
PRL 120-24 C-r001.d	Aflatoxin B2	Sample	4.685	1	ND	
PRL 120-24 C-r002.d	Aflatoxin B2	Sample	5.038	1	ND	
PRL 120-24 D-r001.d	Aflatoxin B2	Sample	5.120	1	ND	
PRL 120-24 D-r002.d	Aflatoxin B2	Sample	5.283	3	ND	
PRL 120-24 E-r001.d	Aflatoxin B2	Sample	5.147	2	ND	
PRL 120-24 E-r002.d	Aflatoxin B2	Sample	5.663	1	ND	
PRL 120-24 F-r001.d	Aflatoxin B2	Sample	5.066	0	ND	
PRL 120-24 F-r002.d	Aflatoxin B2	Sample	4.794	1	ND	
PRL 120-24 G-r001.d	Aflatoxin B2	Sample	5.527	3	ND	
PRL 120-24 G-r002.d	Aflatoxin B2	Sample	4.848	4	ND	
PRL 120-24 H-r001.d	Aflatoxin B2	Sample	4.848	2	ND	
PRL 120-24 H-r002.d	Aflatoxin B2	Sample	4.767	3	ND	
PRL 120-24 I-r001.d	Aflatoxin B2	Sample	4.903	3	ND	
PRL 120-24 I-r002.d	Aflatoxin B2	Sample	5.174	3	ND	
PRL 120-24J-r001.d	Aflatoxin B2	Sample	5.283	2	ND	
PRL 120-24J-r002.d	Aflatoxin B2	Sample	5.066	2	ND	

Table 5: Quantitation Results for Aflatoxin B1

Data File	Compound	Sample Type	RT	Resp.	Final Conc.	Recovery
Blank.d	Aflatoxin B1	Blank	5.588	4	ND	
Aflatoxin STD 1ppb.d	Aflatoxin B1	Calibration	5.534	853	ND	0.00
Aflatoxin STD 5ppb.d	Aflatoxin B1	Calibration	5.561	5052	3.1267	62.53
Aflatoxin STD 10ppb.d	Aflatoxin B1	Calibration	5.588	8201	10.5138	105.14
Aflatoxin STD 25ppb.d	Aflatoxin B1	Calibration	5.561	14992	26.4423	105.77
Aflatoxin STD 50ppb.d	Aflatoxin B1	Calibration	5.561	25391	50.8354	101.67
Aflatoxin STD 75ppb.d	Aflatoxin B1	Calibration	5.588	35381	74.2712	99.03
Aflatoxin STD 100ppb.d	Aflatoxin B1	Calibration	5.561	46269	99.8106	99.81
PRL 120-24 A-r001.d	Aflatoxin B1	Sample	5.806	1	ND	
PRL 120-24 A-r002.d	Aflatoxin B1	Sample	5.317	1	ND	
PRL 120-24B -r001.d	Aflatoxin B1	Sample	5.724	1	ND	
PRL 120-24B -r002.d	Aflatoxin B1	Sample	5.480	0	ND	
PRL 120-24 C-r001.d	Aflatoxin B1	Sample	5.045	1	ND	
PRL 120-24 C-r002.d	Aflatoxin B1	Sample	5.697	1	ND	
PRL 120-24 D-r001.d	Aflatoxin B1	Sample	5.670	0	ND	
PRL 120-24 D-r002.d	Aflatoxin B1	Sample	5.751	1	ND	
PRL 120-24 E-r001.d	Aflatoxin B1	Sample	4.719	1	ND	
PRL 120-24 E-r002.d	Aflatoxin B1	Sample	5.670	1	ND	
PRL 120-24 F-r001.d	Aflatoxin B1	Sample	6.050	2	ND	
PRL 120-24 F-r002.d	Aflatoxin B1	Sample	5.235	1	ND	
PRL 120-24 G-r001.d	Aflatoxin B1	Sample	5.561	1	ND	
PRL 120-24 G-r002.d	Aflatoxin B1	Sample	5.751	4	ND	
PRL 120-24 H-r001.d	Aflatoxin B1	Sample	5.670	3	ND	
PRL 120-24 H-r002.d	Aflatoxin B1	Sample	5.453	0	ND	
PRL 120-24 I-r001.d	Aflatoxin B1	Sample	4.991	2	ND	
PRL 120-24 I-r002.d	Aflatoxin B1	Sample	5.018	2	ND	
PRL 120-24J-r001.d	Aflatoxin B1	Sample	6.485	1	ND	
PRL 120-24J-r002.d	Aflatoxin B1	Sample	5.887	2	ND	

DISCUSSION

This study aimed to assess the levels of aflatoxins in silver cyprinid (silverfish) using the highly sensitive LC-MS/MS analytical technique. The results indicated that no detectable levels of aflatoxins were found in the silverfish samples analyzed, as the final concentration for aflatoxins was not detected. Despite this, the study yielded valuable data regarding the calibration curves and instrument response, showing the methodology's performance and accuracy.

Seven levels of aflatoxin standards were prepared, including concentrations of 1, 5, 10, 25, 50, 75, and 100 ppb. These were injected into the LC-MS/MS, with a consistent injection volume of 1 µL for each sample. The use of seven levels of aflatoxin standards ensured that the calibration curve covered a wide dynamic range[21, 22]. These levels were critical in accurately quantifying aflatoxin concentrations when present, allowing the system to detect very low to moderately high levels of aflatoxin contamination. Each level's response was recorded, and as expected, higher aflatoxin concentrations resulted in larger peak areas. The injection volume of 1 µL provided high sensitivity, during, maintaining consistent peak shapes and retention times across the calibration range. The response of the mass spectrometer was recorded at each concentration level, and calibration curves were generated by plotting the detector's response against the known concentrations of aflatoxin standards. The linearity of these curves was confirmed, with coefficients of determination (R^2 values) exceeding 0.99 for all aflatoxins (B1, B2, G1, and G2)[23, 24].

Experimentally, recovery should be between 70 and 120%. This shows that the extraction method is a suitable one. The acquisition method that was used was established in 2022 and it was called 14-12-2022 Aflatoxin Method[25]. The analysis was carried out on plate 2 of the LC-MS/MS thus the samples had position 2 (P2). To reduce analytical mistakes and guarantee consistency, each sample was examined twice. Aflatoxin-free blank samples were conducted on a regular basis to make sure there was no contamination or holdover from earlier tests. While no aflatoxins were detected in the silverfish samples, the calibration curve analysis and instrumental accuracy demonstrate that the LC-MS/MS method was capable of detecting and quantifying aflatoxins with high precision. The linearity of the calibration curves confirms that the method would have accurately quantified aflatoxins if they were present[26, 27].

The absence of aflatoxins in the silver cyprinid samples indicates that, under the current storage, handling, and environmental conditions in Kansanga, silver cyprinid remains a safe food source free from aflatoxin contamination. This result aligns with findings from other studies that have shown that when proper post-harvest handling practices are followed, the risk of fungal growth and subsequent aflatoxin production is significantly minimized[28]. Silver cyprinid is typically sun-dried after capture, which helps reduce moisture levels, a key factor in the prevention of fungal contamination. The lack of aflatoxins in this study may be attributed to effective drying practices. Drying silver cyprinid in direct sunlight reduces moisture levels to below the critical limit required for Aspergillus fungi to thrive. Vendors in Kansanga may be employing effective drying practices, thus mitigating the risk of aflatoxin contamination[29, 30]. Another factor is proper storage conditions. Although the study did not directly measure storage conditions, it is likely that vendors store their products in low-humidity, well-ventilated environments[30]. This would further prevent the growth of aflatoxin-producing fungi, as high moisture content is a critical factor for fungal proliferation. The absence of aflatoxins in the samples could reflect the relatively dry environmental conditions during the study.

While aflatoxins were not detected in this study, previous research conducted in other regions has reported varying levels of aflatoxin contamination in dried fish and other food products. For instance, studies in East Africa have detected aflatoxins in maize, groundnuts, and sometimes fish, often linked to poor storage and inadequate drying techniques[31]. The differences in contamination levels across regions can be attributed to variations in environmental factors, post-harvest practices, and the level of awareness and training among vendors. In contrast to these reports, the results of this study are reassuring; suggesting that silver cyprinid sold in Kansanga may not share the same risk profile as other regions where aflatoxin contamination is more prevalent or widespread[32, 33]. However, this does not imply that the risk of contamination is non-existent. Continuous vigilance and monitoring are necessary to ensure that food safety standards are maintained. The absence of aflatoxins in the analyzed samples is a positive finding from a public health perspective. Aflatoxins are potent carcinogens, and long-term exposure, even at low levels, can lead to severe health problems, including liver cancer, immune suppression, and stunted growth in children. Since silver cyprinid is a popular and affordable source of protein for many Ugandans, especially in lower-income households, ensuring that it remains free from aflatoxins is critical for safeguarding public health. Although no aflatoxins were detected in this study, there remains a potential risk if environmental conditions or storage practices change. For example, during the rainy season, higher humidity levels may create favorable conditions for fungal growth, potentially leading to future contamination. Therefore, it is important that stakeholders involved in the silver cyprinid

trade continue to adopt best practices in handling, drying, and storage to prevent any contamination.

CONCLUSION

The study aimed to assess the levels of aflatoxins in silver cyprinid sold in Kansanga, Kampala, using the LC-MS/MS method. Based on the results of the analysis, no detectable levels of aflatoxins were found in any of the samples collected from various vendors. This finding suggests that, under the prevailing conditions in Kansanga, the risk of aflatoxin contamination in silver cyprinid is minimal. Several factors may have contributed to this, including proper drying techniques, appropriate storage practices, and favorable environmental conditions that discourage the growth of aflatoxin-producing fungi. The absence of aflatoxins in the analyzed silver cyprinid samples is a positive finding for public health and food safety, indicating that the silver cyprinid sold in this area meets food safety standards. However, it is essential to recognize that this is a single study conducted over a limited timeframe, and the risk of aflatoxin contamination could still arise under different circumstances, such as changes in handling, storage, or environmental factors.

Recommendations

Although aflatoxins were not detected in this study, regular monitoring of silver cyprinid and other food products for aflatoxin contamination should continue. Periodic testing using methods such as LC-MS/MS will help ensure that silver cyprinid remains safe for consumption and that potential contamination is identified early. Vendors and consumers should be educated on the importance of proper handling and storage practices to prevent aflatoxin contamination. Emphasizing practices such as thorough drying and storing silver cyprinid in low-humidity, well-ventilated areas can help maintain food safety in the long term.

Though the study results are encouraging, regulatory authorities should continue to enforce and strengthen food safety standards for aflatoxins in Uganda. Implementing guidelines on drying, storage, and handling across markets will help mitigate future risks, especially in more humid or poorly managed environments. Additional research is recommended to assess aflatoxin levels in other food products sold in Kansanga and surrounding areas. Comparative studies could also examine seasonal variations, changes in storage conditions, or other factors that might affect aflatoxin contamination. Vendors should be supported with resources and knowledge to implement best practices in food storage and hygiene. The provision of better storage facilities, especially in wet or hot seasons, would further reduce the risk of fungal growth and aflatoxin production.

ABBREVIATIONS

EAC:	East African Community
HBV:	Hepatitis B Virus
HCC:	Hepatocellular Carcinoma
AFB1:	Aflatoxin B1
AFB2:	Aflatoxin B2
AFG1:	Aflatoxin G1
AFG2:	Aflatoxin G2
AFM1:	Aflatoxin M1
AFM2:	Aflatoxin M2
ppm:	Parts per million
ppb:	Parts per billion
GC:	Gas Chromatography
TLC:	Thin Layer Chromatography
LC:	Liquid Chromatography
HPLC:	High Performance Liquid Chromatography
RP-HPLC:	Reversed-Phase High Performance Liquid Chromatography
FLD:	Fluorescence Detection
MLs:	Maximum Limits
EU:	European Union
ELISA:	Enzyme-Linked Immunosorbent Assay
EFSA:	European Food Safety Authority
LC-MS/MS:	Liquid Chromatography-Tandem Mass Spectrometry
RT:	Retention time
Resp.:	Response

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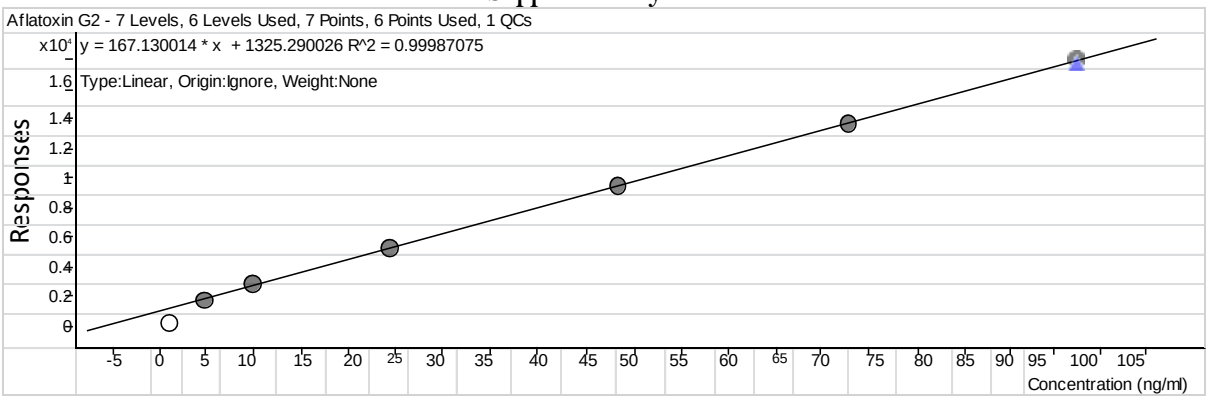
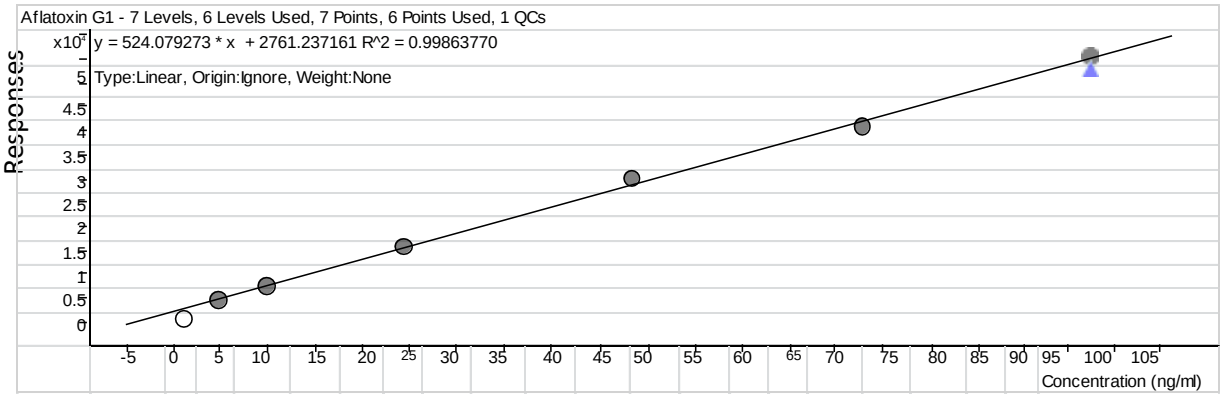
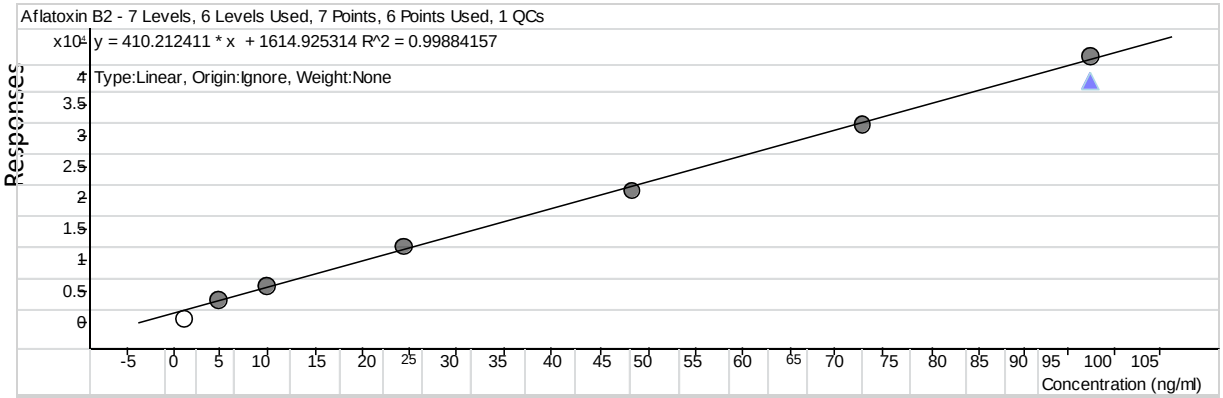
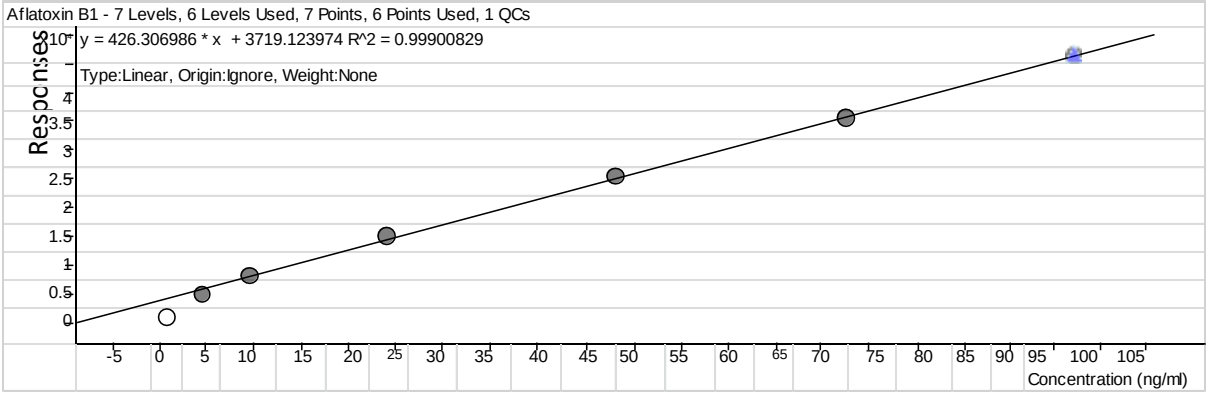
Supplementary data









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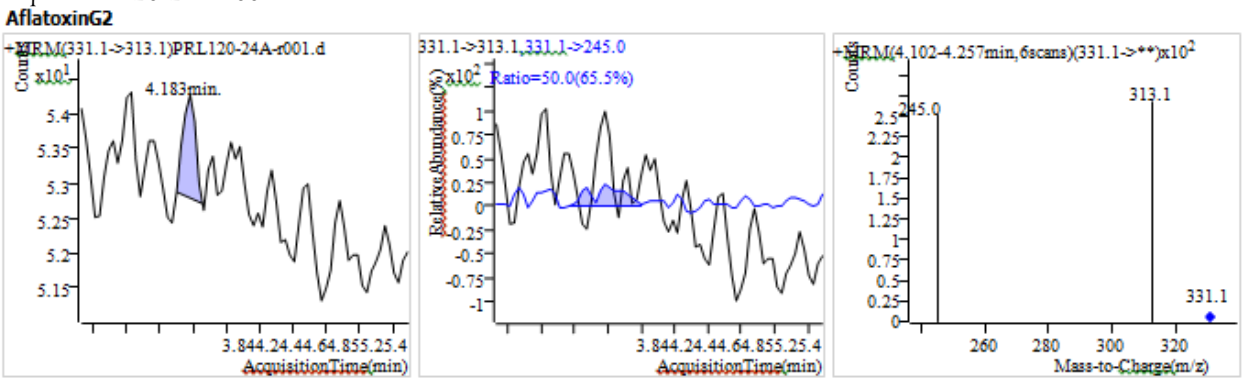

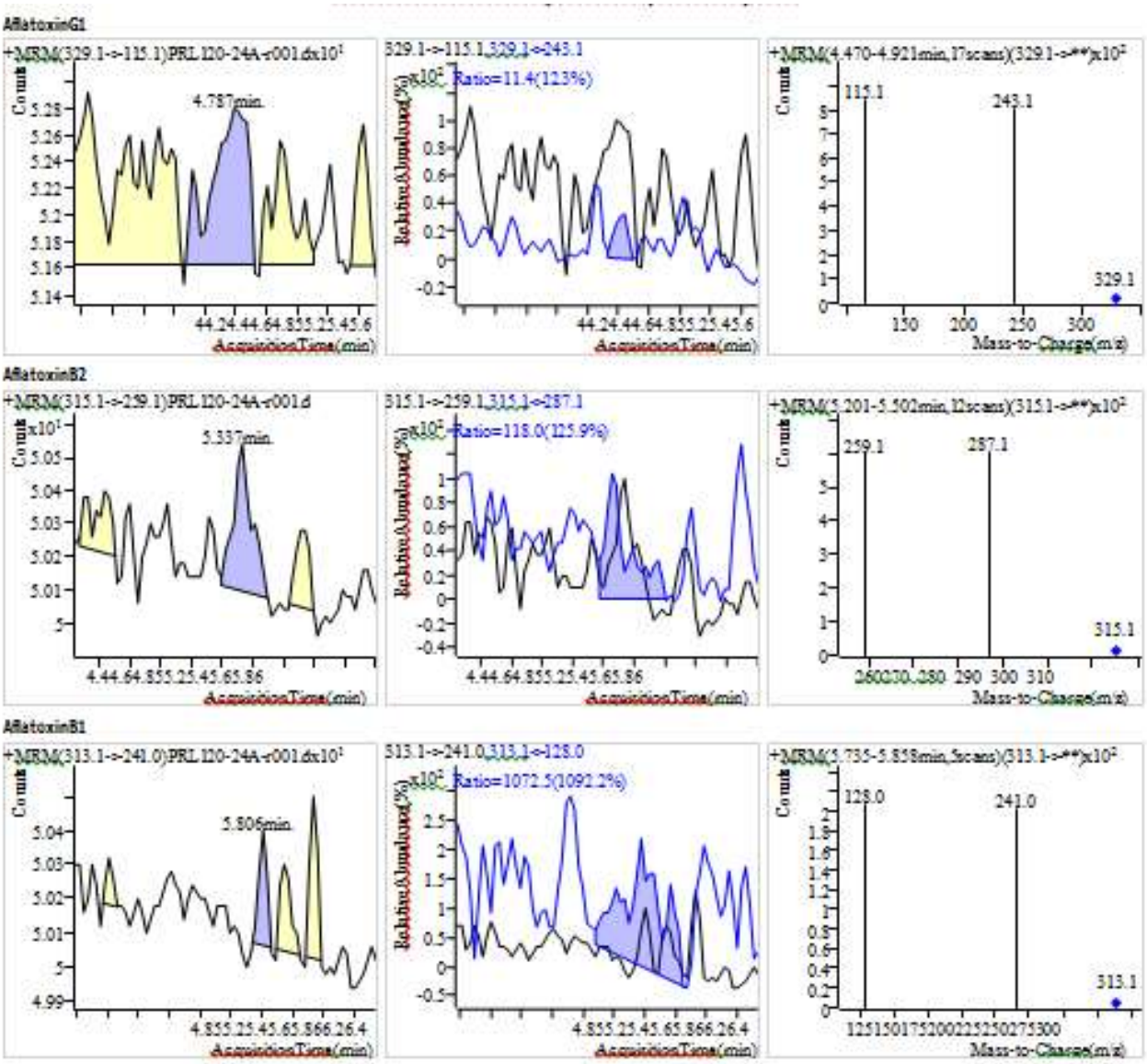

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