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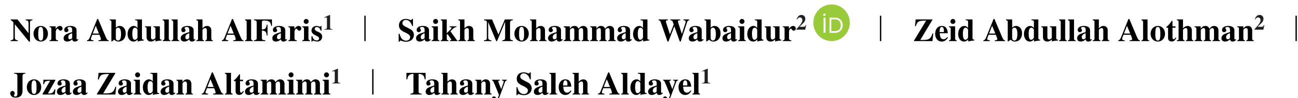
RESEARCH ARTICLE

Fast and efficient immunoaffinity column cleanup and liquid chromatography–tandem mass spectrometry method for the quantitative analysis of aflatoxins in baby food and feeds



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An ultra high performance liquid chromatography-tandem mass spectrometric method has been developed for the highly sensitive and selective determination of regulated aflatoxins. The extraction of aflatoxins from baby food matrices were performed using liquid–liquid extraction procedure followed by immunoaffinity column cleanup. The higher sensitivity for the determination of target aflatoxins was fulfilled by applying a preconcentration step with immunoaffinity columns after acetonitrile–water extraction. The enhanced selectivity was attained with the triple quadrupole mass analyzer operated in electrospray positive ionization mode. Method validation was tested in five different baby food matrices by recovery experiments. Satisfactory recoveries, between 92 and 103%, with relative standard deviations lower than 8% were achieved in all the tested matrices. The proposed method was found to be specific as no interference peaks were observed for blank samples. The limit of detection of the method was found to be in the range of 0.003–0.008 ng/mL. The validated method was fruitfully applied to the screening of aflatoxins in baby foods and feeds sample retailed in local markets of Riyadh, Saudi Arabia. The obtained levels of all analyzed aflatoxins were below the regulation limits set by European Agency.

KEYWORDS
aflatoxins, baby food, mass spectrometry, ultra high performance liquid chromatography

1 | INTRODUCTION

Aflatoxins are lethal secondary metabolite formed by the organism of the fungi family *Aspergillus* species including *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* [1,2]. These are the poisonous chemical that willingly colonize crops and cause infection to humans and other animals [3]. Cereals, for instance, wheat, rice, maize, chickpeas, and beans are the source of several diets worldwide. They have widely been used in production of baby foods providing the baby possible energy and nutrients, and nourishing their anticipated growth and development. The Food Agriculture Organization has reported that a large quantity of cereals have been used for human consumption including baby foods and feeds, and among them at least 25% are contaminated [4]. The lack of experiences in mycotoxins application by the growers for killing the pest and increasing the food quantity seriously contaminates the food and feed products and among them grains are the most affected kind of food. Such contamination has high impact toward human health and mostly on the children due to their higher metabolic rate and lower ability to detoxify such substances [5,6]. At present, analysis of various mycotoxins is still a main threat in the control and assessment of food products. Among various mycotoxins, aflatoxins, AFB1,

Article Related Abbreviations: BEH, ethylene bridged hybrid; IAC, immunoaffinity column; MRM, multiple reaction monitoring.

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AFB2, AFG1, and AFG2 are most commonly found in cereal products, cereals, spices, nuts, beer, dry fruits, and baby food [7]. Among them, AFB1 is reported to be the most highly toxic for humans and its ingestion cause serious health hazards including, cirrhosis, acute liver cancer, and hepatic necrosis [8]. The World Health Organization in 1993 and the International Agency for Research on Cancer (IARC) have categorized AFB1 as a Group 1 carcinogen to human, and other three aflatoxins have been recognized as Group 2B carcinogens [9,10]. Hence, to protect the community, the European Union food safety legislation has strictly set the latest regulations for lower limits of aflatoxins levels in certain food and feeds [11].

Different features play a key character in the decision-making process of aflatoxins limits setting including scientific factors and socioeconomic concerns [12]. The regulation establishes maximum levels of aflatoxins as low as can be achievable [11]. Exceptional attention has been given in food for infants and children. Infant and young children formulas are consumed by individuals with low bodyweight and the toxic effect of aflatoxins in children will be more significant than in grown person as they have higher metabolic rate and low resistivity power to decontaminate hazardous materials [5]. Furthermore, these food products are made from cereals, which are one of the items where aflatoxins emerge in relatively larger amounts and frequencies than in other food products. The European Union has set the strictest maximum limits for aflatoxins, AFB1 for baby foods, and processed cereal-based foods and the limit is 0.1 μg/kg [7,11]. During growth, processing, storage, and transport of food and feed items, there are huge possibilities for contaminants or residues predominantly produced into them as there are high probability of growing the fungi (*Aspergillus* species) producing aflatoxins, especially in the hot climatic region [13].

In order to achieve the very low regulation levels of aflatoxins established by the European Commission, highly sensitive and reliable analytical methods are in high demand. Various techniques have been used for rapid aflatoxins analysis earlier, requiring additional confirmatory analysis due to unacceptable quantification accuracy [14]. The LC coupled to different screening principles has become the most important technique for the quantitation of aflatoxins in various food and feeds [3, 15]. Among them, HPLC–fluorescence detection has been used as the preferred approach for aflatoxins analysis, however, such method shows limitations as it requires a variety of cleanup protocols [16].

LC-MS/MS has been reported and considered as the universal approach for mycotoxin screening due to their high selectivity for analyzing several analyte in one run [17–22]. Recently, a few multi-mycotoxin analysis methods have been described in varied food commodities, however the validated methods for baby food analysis are rare as high sensitivity is required to satisfy the strict regulation in such types of matrices [7,23]. To achieve such a low regulation limits, cleanup and/or enrichment techniques are usually required to determine aflatoxins in baby food and feeds. It was obvious that the use of SPE with multifunctional cartridges is an interesting approach to achieve such target [24]. The immunoaffinity columns (IAC) were also designed and used earlier considering their high specificity and selectivity for selected aflatoxins analysis [25,26]. For multiresidual applications, introduction of IAC columns with specific antibodies has been used as an interesting alternative approach as they reduce the sample throughput and allow simultaneous quantitation [27].

The application of UHPLC has permitted faster and efficient separations for many target compounds. It produces narrower peaks with superior sensitivity and peak resolution. The UHPLC in combination with MS has also been reported for the quantitation of mycotoxins [1,7]. The UHPLC-MS/MS in multiple reaction monitoring (MRM) mode is considered as one of the most selective and sensitive analytical methods for quantification and confirmation of organic pollutants and residues in different food matrices [7]. The research relating to the occurrence of aflatoxins analysis in baby food from the Kingdom of Saudi Arabia has been a challenge. Therefore, in the current study, the aflatoxins were extracted from various baby food and feeds using aqueous-organic extraction followed by IAC cleanup, and the presence and possible levels of them were determined using UHPLC-MS/MS method with satisfactory results.

2 | MATERIALS AND METHODS

2.1 | Reagents and chemicals

Sigma Aldrich (St. Louis, MO, USA) provided individual standards of aflatoxins (AFB1, AFB2, AFG1, and AFG2). The chemical structures of analyzed aflatoxins are provided in Figure 1. All standards have purity higher than 99%. Milli-Q water was obtained from Millipore water purification system (Bedford, MA, USA). Analytical-grade acetonitrile (CH_3CN) and methanol (CH_3OH) were acquired from Scharlab (Barcelona, Spain). Formic acid (HCOOH, purity > 98%) was obtained from Fluka (Buchs, Switzerland). Mix solutions of aflatoxins of 100 mg/L, were prepared diluting reference standard in certain volume of acetonitrile. Working standard solutions for calibration and spiking samples were prepared by diluting the stock solution (100 μg/L) with mobile phase. All the stock and intermediate standard solutions were stowed in a refrigerator at 4°C.

2.2 | Instrumentation and MS conditions

The chromatographic analysis of aflatoxins was performed on a Waters Acquity UPLC system furnished with a quaternary pump (Milford, MA, USA). The separations of

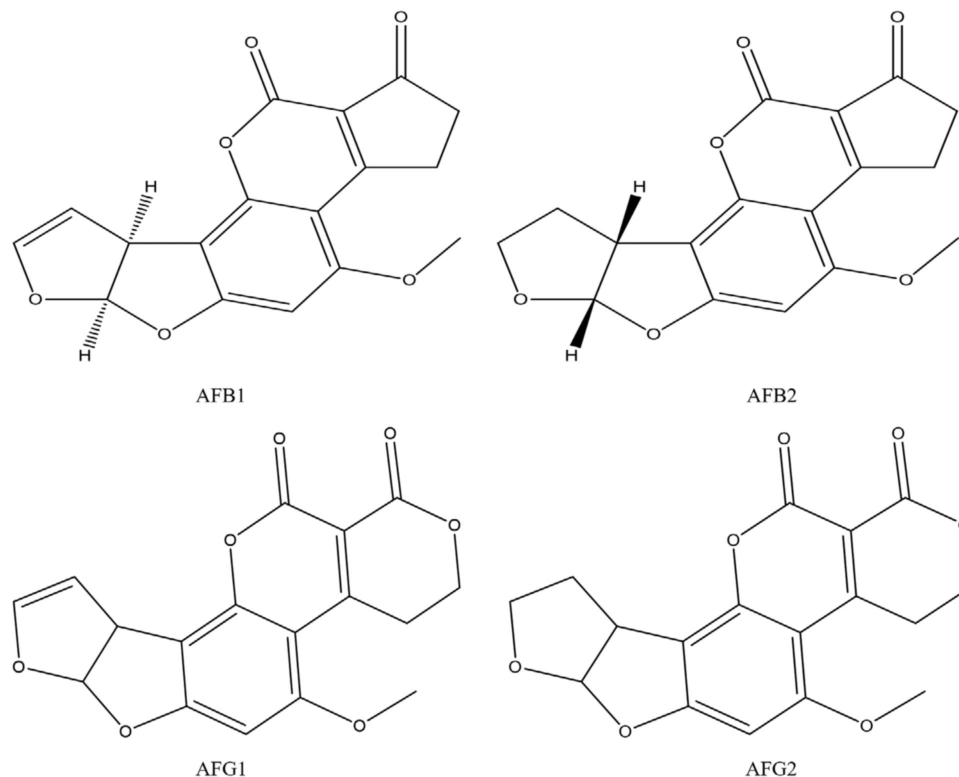

FIGURE 1 Chemical structures of aflatoxins, AFB1, AFB2, AFG1, and AFG2

the target analytes were achieved using an Acquity ethylene bridged hybrid (BEH) C_{18} column of dimension 100 mm × 2.1 mm id, and 1.7 µm particle size (Waters, Milford). The binary mobile phase of different compositions of methanol and aqueous formic acid (0.1%, v/v) was used during the analysis at 0.4 mL/min flow. The immunoaffinity column (Aflaochra HPLCTM) was supplied by Vicam (Tecasa, Madrid, Spain). Quattro Premier triple quadrupole mass spectrometer (Micromass, Milford, MA, USA) equipped with ESI Z spray source was used for mass spectrometric analysis. The protonated molecular ions of each aflatoxins were used as a precursor ion. Nitrogen (purity, 99.99%) was supplied by Peak Scientific (model NM30LA nitrogen generator, Inchinann, UK) was used as nebulizing and drying gas. High-purity argon (99.999%) supplied by Specialty Gas Centre (Jeddah, Saudi Arabia) was used as collision gases. The target vacuum needed for thee MS was achieved from An Oerlikon rotary pump (SOGEVAC SV40 BI, France). Data acquisition and analysis were carried out by MassLynx V4.1 software. Turbo Evaporator (Zymark, Hoptkinton, USA) was used for drying the extracted samples.

2.3 | Samples

Nineteen baby food samples including five powdered milk, five milk with cereals, two flavored milk, five cereals infant formula, and two raw milk were analyzed during the experiments, which were sold in different supermarkets and pharmacy stores of Riyadh (Saudi Arabia). Powdered milk was prepared using water according to the instruction of manufacturer in order to determine aflatoxins levels in the product ready to use [11].

2.4 | Analytical procedure

For solid baby food, the samples were accurately weighed (10 g) and transferred to a 50-mL polypropylene centrifuge tubes. The powder milk for babies was prepared using water according to the manufacturer instructions, where the maximum levels referred in products ready to use by the European Regulation. The possible aflatoxins contaminants of the samples were extracted with 40 mL acetonitrile–water (78:22) by vigorous shaking on a mechanical shaker (IKA KS 260 basic) for 120 min. Then extracts were centrifuged individually with Hermle Labortechnik Centrifuge (Germany) at 5000 rpm for 8 min, and the 10 mL of the supernatant for each sample was separated and collected for further experiments. For liquid samples such as raw milk, milk with cereals, and reconstituted powdered milk, the samples were precisely weighed (8.5 g) and poured into a 50-mL centrifuge tube individually. Then, 30 mL of acetonitrile was added to liquid samples to obtain final composition of acetonitrile–aqueous (78:22) as used for the extraction of aflatoxins from the solid matrices. During this process, the protein content was precipitated and the whole mixture was shaken using a mechanical shaker for 120 min. Finally, the mixture solutions were centrifuged

at 5000 rpm for 8 min, and 10 mL of the supernatant was collected and stored in refrigerator at 4°C until their analysis.

For the immunoaffinity cleanup, first the acetonitrile contents of the stored extract (10 mL) were removed by using a turbo evaporator system (water bath at 50°C under moderate nitrogen stream) followed by dilution with Milli-q water up to 10 mL mark. Then the whole amount of the aqueous extracts was passed through an IAC Aflaochra column at a flow speed of one/two drops per second. After passing all the aqueous extracts, the column was washed passing with 5–10 mL of Milli-q water. Finally, the target analytes were eluted from the IAC column with 4–5 mL methanol at a flow rate of 1–2 drops/s. The elutes were then dried under the influence of mild nitrogen stream at 48°C and the dry sample was then reconstituted with mobile phase, a mixture of methanol/0.1% aqueous formic acid (75:25, v/v) to a final volume of 1.0 mL. To circumvent any hindrance during the UHPLC injection, microfiltration with a 0.22 µm PVDF syringe filter (Membrane Solutions, TX, USA) was performed before injecting into the system. Five microliter of this final solution was injected into the UHPLC-MS/MS system. The quantification of aflatoxins was performed by plotting external calibration curve with standards.

2.5 | Validation study

The developed UHPLC-MS/MS method was validated for the selected aflatoxins in all five different matrices of the real samples (powdered milk, milk with cereals, flavored milk, cereals infant formula, and raw milk). In order to confirm the method quality, the following parameters were evaluated in terms of linearity, day-to-day and run-to-run precision, specificity, LOD, and LOQ. Seven-point calibration plot of standard concentration versus peak area in triplicate was constructed to find out the linearity of the proposed method. Repeatability and reproducibility of the method were determined by recovery experiments, analyzing aflatoxins fortified samples in triplicate. This experiment was performed at concentration levels of 0.1 ng/mL for cereals infant formula and at 0.025 ng/mL for raw milk and milk products. The LOD and LOQ were calculated from the chromatograms of the samples spiked at the lowest validation concentration for an S/N of 3 and 10, respectively.

3 | RESULTS AND DISCUSSION

3.1 | UHPLC method optimization

Different chromatographic parameters were optimized to achieve best peak of the target analyte. At first, RP BEH columns with C_4, C_8, and C_{18} functionality were tested for chromatographic analysis. Among the investigated column functionalities, RP BEH C_{18} column was showed high signal intensity with sharper peaks of reproducible area at varying concentration levels of analytes. The special proprietary end-capping design of BEH columns delivers higher efficiencies, highest MS sensitivities, and sharp peaks during the analysis. Acquity® UPLC HILIC (hydrophilic interaction liquid chromatography) column was investigated as well but the better separation was achieved with the precursor one. The mobile phase has notable effects on detector response in terms of the peak symmetry and intensity. The binary mobile phase including water–methanol, aqueous formic acid (0.1%)–methanol, and acetonitrile–water with and without formic acid with changing compositions were tested and best results were obtained with methanol/aqueous formic acid (75/25, v/v). Efficient separation of all aflatoxins was achieved under isocratic elution mode in less than 4 min (Figure 2). Hence, this binary mobile phase composition was selected for determination of aflatoxins with satisfactory separation of the peaks. All analyzed aflatoxins were appeared on the chromatogram according to their degree of polarity [28] and the retention time for AFB1, AFB2, AFG1, and AFG2 was 3.65, 3.25, 2.65, and 2.04 min, respectively (Figure 2).

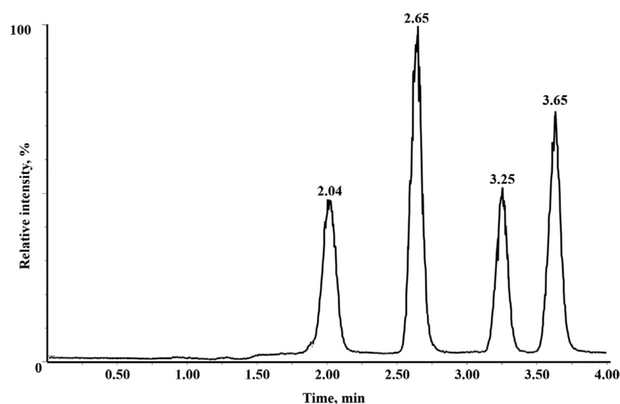

FIGURE 2 UHPLC–MS/MS chromatograms of aflatoxins (AFB1: 3.65 min; AFB2: 3.25 min; AFG1: 2.65 min; AFG2: 2.04 min), corresponding to 25 ng/L mixed standard solution after extraction procedure

3.1.1 | MS conditions optimization

Ionization conditions for all analyzed aflatoxins were obtained by their direct infusion into the electrospray source (ESI) of the MS detector. Both positive and negative ionization mode were operated for highest relative intensity. The negative operation mode of the ionization source was unable to produce intense signal of the analyte, while the positive ionization mode successfully produced intense peak of protonated molecular ion of each analyte (precursor ion). Hence, further experiment was carried in positive ESI mode. During the mass spectrometric analysis, cone voltage was optimized to obtain the precursor ion and the product ion spectra were

TABLE 1 Parameters of multiple reaction monitoring transitions for each aflatoxins using UHPLC-MS/MS

Mycotoxins	Retention time (min)	Parent ion $[M + H]^+$, m/z	Quantification transition		Confirmation transition	
			Daughter (m/z)	Collision energy, eV	Daughter (m/z)	Collision energy, eV
AFB1	3.65	313	282	17	198	21
AFB2	3.25	315	284	20	228	23
AFG1	2.65	329	298	20	210	26
AFG2	2.04	331	300	18	212	28

acquired at different collision energies. The optimized experimental conditions achieved during the tuning of aflatoxins were as follows: cone voltage, 23 V; capillary voltage, 3.2 kV; source temperature, 130°C; cone gas flow, 50 L/h; desolvation gas temperature, 300°C; desolvation gas flow, 600 L/h; collision gas flow, 0.25 mL/min. Considering the higher sensitivity and selectivity showed by MRM mode, it was chosen for the analysis of all target aflatoxins. The transition with higher peak response was selected for quantitative analysis purposes, and the transition with low peak area was chosen for qualitative analysis of the analytes. The optimized MRM parameters including selected transitions for aflatoxins have been listed in Table 1.

3.2 | Purification of samples

By direct injection of the extracted sample into the chromatographic system, it was quite impossible to determine the low analyte levels tested. Therefore, the IAC column cleanup was used for purification of the aflatoxins reducing the matrix interferences as mentioned in section 2.4. The target aflatoxins bound to the respective antibody of the IAC were eluted with 4–5 mL methanol, reconstituted, and acquired in HPLC vials. During this, the methanol passed within 2 min in order to complete release of the toxins that are attached inside the affinity column. Finally, the dried elute was dissolved in 1 mL of optimum mobile phase, filtered through 0.22 μm PVDF syringe filter, and stored at 4°C in a refrigerator until their analysis.

The efficiency of the acetonitrile–water extraction and the IAC was evaluated in terms of recovery study. To avoid matrix interferences, the IAC cleanup method was optimized in terms of the loading flow, column washing, and elution solvents. The high flow rate of the sample loading might affected the IAC recovery. So various loading rate was checked and best recovery was found for the flow rate of one/two drops per second. To decrease the background noise of the chromatogram, the column was washed repeatedly with sufficient amount of water, and lower RSDs were achieved with 5–10 mL of water washing. Sample elution condition was also optimized and methanol with various water compositions were tested and best recovery was found using 4–5 mL pure methanol and was chosen as elution solvent. Using the optimum parameters, the recoveries for standard aflatoxins subjected to the sample treatment followed by cleanup process were approximately 100%. Afterward, the column performances were evaluated for a nonfortified and three fortified real samples, which were analyzed in triplicates. The recoveries of AFB1, AFB2, AFG1, and AFG2 were found to be in the range of 93–107% with RSDs less than 3.2% for triplicate measurements. The chromatograms of nonspiked and spiked samples were satisfactory, indicating complete separation from other detectable constituents.

3.3 | Method validation

The proposed method was validated in terms of linearity, detection, and quantification limits and precision before applying it to real sample analysis. Linear calibrations were constructed for all compounds and were found in the range of 0.01–10.0 ng/mL with the correlation coefficient (r^2) higher than 0.994 for all the analyzed aflatoxins. Accuracy of the method was estimated by spiking blank samples ($n = 5$) at the same levels established by the European legislation in baby food and milk derivate. The sample was spiked at 0.10 ng/mL and the liquid samples were spiked at 0.025 ng/mL levels. Satisfactory recovery results (92–103%) were obtained for all analyte/matrix combinations tested, with RSDs lower than 8%. Considering the analysis difficulties level, the results were very satisfactory. The proposed method was found to be specific as no interference peaks were observed for blank samples at the same retention window of the standard chromatogram.

Sensitivity was evaluated of the proposed UHPLC-MS/MS method, LOD and LOQ were calculated based on S/N of three and S/N of 10, respectively, from the chromatograms of the injected samples spiked at the lowest validation level. The calculated LOD and LOQ for the method were found to be in the range of 0.003–0.008 ng/mL and 0.01–0.025 ng/mL for all the aflatoxins, respectively (Table 2).

For run-to-run precision (repeatability), five replicates of the extraction experiment were carried out at level of 0.5 ng/mL AFB1, AFB2, AFG1, and AFG2 mixture at same day, and to evaluate day-to-day precision (reproducibility), 15 replicates of the analysis were executed with the same solutions over three consecutive days (five replicates each day).

TABLE 2 Linear regression data, LOD, LOQ, and precision of aflatoxins by the proposed method

Aflatoxin	Range (ng/mL)	Regression line		r^2	LOD[a] (ng/mL)	LOQ[b] (ng/mL)	Precision[c] (RSD, %)	
		Slope	Intercept				Run-to-run	Day-to-day
AFB1	0.01–10.0	11.94	–25.07	0.994	0.006	0.019	3.9	4.3
AFB2	0.02–10.0	6.57	82.35	0.997	0.004	0.013	3.7	4.5
AFG1	0.01–10.0	31.54	–60.07	0.996	0.008	0.025	2.9	4.9
AFG2	0.02–10.0	12.10	–50.96	0.994	0.004	0.014	2.8	5.2

r^2: Correlation coefficients.
[a] S/N = 3/1.
[b] S/N = 10/1.
[c] $n = 5$.

TABLE 3 Aflatoxins level in baby food samples ± SD (μg/kg) and recovery (%)

Sample code	Samples name	AFB1± SD (%R)	AFB2 ± SD (%R)	AFG1 ± SD (%R)	AFG2 ± SD (%R)
S1	Powder milk	nd (93)	nd (93)	nd (92)	nd (92)
S2	Powder milk	nd (95)	nd (92)	nd (95)	nd (93)
S3	Powder milk	nd (99)	nd (94)	nd (97)	nd (93)
S4	Powder milk	nd (97)	nd (93)	nd (96)	nd (92)
S5	Powder milk	nd (93)	nd (95)	nd (92)	nd (94)
S6	Milk with cereals	0.076 ± 0.005 (103)	nd (97)	nd (94)	nd (94)
S7	Milk with cereals	0.092 ± 0. 003 (101)	nd (98)	nd (97)	nd (93)
S8	Milk with cereals	nd (98)	nd (94)	nd (95)	nd (93)
S9	Milk with cereals	nd (97)	nd (92)	nd (95)	nd (93)
S10	Milk with cereals	nd (98)	nd (95)	nd (94)	nd (90)
S11	Flavored milk	nd (97)	nd (94)	nd (94)	nd (92)
S12	Flavored milk	nd (95)	nd (92)	nd (95)	nd (96)
S13	Cereals infant formula	0.086 ± 0.003 (103)	nd (92)	nd (92)	nd (92)
S14	Cereals infant formula	0.083 ± 0.003 (100)	nd (93)	nd (92)	nd (97)
S15	Cereals infant formula	0.096 ± 0.005 (101)	nd (97)	nd (92)	nd (93)
S16	Cereals infant formula	nd (94)	nd (92)	nd (94)	nd (93)
S17	Cereals infant formula	nd (92)	nd (97)	nd (94)	nd (93)
S18	Raw milk	nd (97)	nd (93)	nd (96)	nd (93)
S19	Raw milk	nd (96)	nd (95)	nd (97)	nd (93)
Total amount of AFB1 found/Mean* of AFB1				0.453/0.091	
Aflatoxins positive samples (%)				5/26.13	

*Mean concentration of AFB1 in detected five samples.

Excellent values for repeatability and reproducibility were attained with RSDs lower than 5.2% in all cases (Table 2). This confirms that the proposed UHPLC-MS/MS method can be successfully applied for accurate and routine analysis of AFB1, AFB2, AFG1, and AFG2. In addition, the efficient IAC cleanup allowed working using standards without relevant matrix effects that normally interrupt the UHPLC–MS/MS methods.

3.4 | Application to samples

The optimized UHPLC-MS/MS method was applied to the analysis of AFB1, AFB2, AFG1, and AFG2 in retailed baby food products purchased from local hypermarkets and pharmacy stores in Riyadh. In total, 19 baby food and feed samples were analyzed and only five samples (26.13%) showed AFB1 contaminations, where three of them were related to baby cereals infant formulas and other two corresponding to milk with cereals. The maximum AFB1 contamination was found at a level of 0.096 μg/kg (S_{15}). Other analyzed aflatoxins were not found in any real samples (Table 3). No other aflatoxins were detected in any samples. The chromatograms for aflatoxins positive samples are shown in Figure 3. Standard addition quantification method was utilized to evaluate the applicability of the method. During this, two nonfortified and five fortified real samples were analyzed in triplicate for each

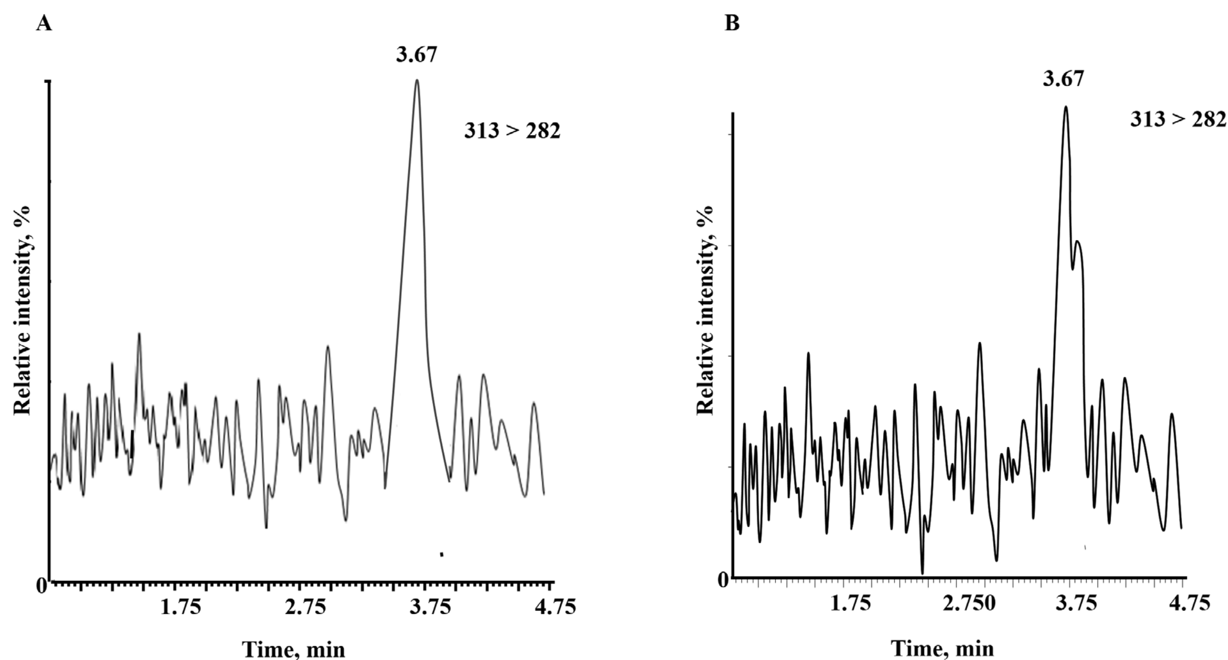

FIGURE 3 UHPLC-MS/MS chromatogram of (A) baby cereals infant formulas and (B) and milk for babies

TABLE 4 Performance comparison of some recent studies on the determination of aflatoxins

Detection method	Preconcentration method	Type of matrices	LOD	RSD (%)	Recovery (%)	Reference
LC-MS	Liquid–liquid extraction	Bean, maize, rice, and wheat	–	–		[15]
LC-fluorescence	Liquid–liquid extraction	cereals	0.003 µg/kg	5.3	>95	[16]
LC-MS/MS	QuEChERS	Menthae haplocalycis	0.007–0.012 µg/kg	0.34 - 10.3	67.1–103	[17]
LC-MS/MS	Liquid–liquid extraction/IAC	Animal feed	0.12 µg/kg	–	78–120	[18]
UHPLC-MS/MS	Liquid–liquid extraction/IAC	Baby food and feeds	0.003–0.008 µg/L	<8	92–103	This work

–, not mentioned.

baby food samples. The recoveries of aflatoxins were found to be in the range of 92–103%. It was observed that the levels of lonely detected aflatoxins (AFB_1) were found to be lower than maximum worldwide permitted levels (0.1 µg/kg) set by European Union [11]. The proper optimizations of IAC cleanup and chromatographic methods were provided negligible matrix effect as the chromatogram of the real samples does not modify the signal. The developed method was compared with the reported method for aflatoxins analysis in terms of sensitivity, matrix types, and RSD (%), as reported in Table 4.

4 | CONCLUDING REMARKS

A highly sensitive and fast UHPLC–MS/MS method has been described for the simultaneous quantitation of aflatoxins in baby food and feeds. The IAC cleanup approach has been found to be effective to minimize matrix interferences and to provide the required selectivity and sensitivity to the developed method. The excellent performance of the method including shorter run time with improved sensitivity comes from different relevant features. The immunoaffinity cartridges consisted of specific antibodies, which are selective toward particular aflatoxins and has made it possible for the simultaneous determination of all targeted aflatoxins in a single run. Additionally, the MS/MS with triple quadrupole analyzer was provided good sensitivity and reliable confirmation of detected aflatoxins in samples. The developed method was validated in different matrices at low concentration levels. The obtained results were highly satisfactory in terms of accuracy and precision in all tested matrices. The recoveries of aflatoxins in all the analyzed samples were in the range of

92–103%. The levels of aflatoxins in all the positive samples were found to be below the regulatory limits. The analyzed results suggested that the developed method is highly sensitive (Table 4) and could be successfully applied for the analysis of aflatoxins even at the trace concentration level in various matrices.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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