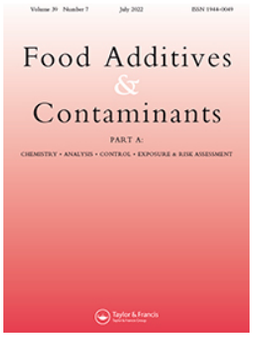

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Quantitative analysis and carcinogenic/non-carcinogenic risk assessment of aflatoxin M_1 in milk-based baby food and infant formula milk – a case study in Iran

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ABSTRACT

In this study, solid-phase extraction (SPE) combined with the dispersive liquid–liquid microextraction based on novel hydrophobic deep eutectic solvent (DLLME – DES) has been developed as an ultra-pre-concentration technique for the extraction of aflatoxin M_1 (AFM1) in milk-based baby food (MBBF) and infant formula milk (IFM) samples followed by HPLC combined with fluorescence detection (HPLC – FL). In addition, carcinogenic and non-carcinogenic risk assessment was performed by health-related risk factors including liver cancer risk (LCR), margin of exposure (MOE) and target hazard quotient (THQ) were calculated using the mean of AFM1 in different infant food samples. The results of the study showed that the mean of AFM1 was statistically significant different between various brands and types of IFM and MBBF. The results of the study showed that the percentage of positive samples higher than the allowable limit of AFM1 in 36 samples of domestic infant formula milk (DIFM), 24 samples of imported infant formula milk (IIFM), 36 samples of domestic milk-based baby food (DMBBF) and 18 samples of imported milk-based baby food (IMBBF) were 41.6, 12.5, 66.7 and 33.3%, respectively. In addition, estimated values for health risk-related factors including LCR, MOE and THQ indicated that for most infants less than one-year-old were higher than the acceptable levels. Based on the results, it can be concluded that the quality of IFM and MBBF consumed in Iran in terms of AFM1 is poor. Therefore, it is necessary to take appropriate measures to reduce the amount of AFM1 in DIFM and DMBBF, and in addition, the IIFM and IMBBF should be controlled qualitatively before supplying the market.

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Aflatoxin M1 solid-phase extraction; dispersive liquid–liquid microextraction; deep eutectic solvent; health risk assessment milk-based baby food; infant formula milk

Introduction

Aflatoxins are a very important group of mycotoxins produced by the main fungi of *Aspergillus* (Tola and Kebede 2016) and aflatoxin M_1 (AFM1) is the most important metabolite among them (Hooshfar et al. 2020). Today, contamination of food, especially livestock and agricultural products with various aflatoxins is one of the most important problems (Guo et al. 2016). When animal feed is contaminated with AFB1, it is metabolised to AFM1 in the liver of the animal 12–24 h later and reaches its maximum after a few days (Bakirci 2001). To prevent AFM1 from entering the human food chain through milk, AFB1 must be prevented from entering animal feed before doing anything. For example, one way to reduce aflatoxin levels in milk and dairy products is to use calcium montmorillonite clay (Maki, Haney, Wang, Ward, Bailey, 2017; Maki, Haney, Wang, Ward, Rude, et al. 2017). In some

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cases, proper packaging and storage of animal feed can be effective in reducing aflatoxins.

In Iran, there are several studies on the amount of AFM1 in milk in different places (Ghaffarian Bahraman et al. 2020; Khaneghahi Abyaneh et al. 2020). Although all people at different ages are affected to varying degrees by the effects of AFM1, people at younger ages, especially children and infants, are more sensitive (Meucci et al. 2010). Consumption of milk and dairy products is very high in all age groups, especially in children, and the presence of AFM1 in these products has been proven in different parts of the world (Tsakiris et al. 2013; Shuib and Saad 2022). In addition, milk-based baby food (MBBF) and infant formula milk (IFM), which are, the important products manufactured from milk are contaminated with AFM1 (El-Tras et al. 2011; Sharafi et al. 2022). The National Standard Organisation (NSO) of Iran has established the maximum limit of AFM1 at 0.25 µg/kg for MBBF and IFM (ISIRI 2002), while the maximum limit of AFM1 in these products in the EU and USA are 0.25 and 0.50 µg/kg, respectively (FDA, CPG 2005; Commission Regulation (EC) 2006). HPLC equipped with a fluorescence detector has been widely used to analyse aflatoxins in various matrices (Scaglioni et al. 2014). However, due to the matrix complexity of MBBF and IFM and the very small amounts of analyte in these samples, a sample pre-treatment and pre-concentration step is necessary before sample analysis (Pirsaheb et al. 2017). The best way to clean-up and eliminate interferences is to use a solid phase extraction (SPE) cartridge. Combination of SPE and dispersive liquid–liquid microextraction based on deep eutectic solvent (DLLME−DES) lead to improve the selectivity of the sample preparation process, high enrichment factor and reduce the achieved LODs for complex matrices. In this research, the pre-concentration and analysis of AFM1 in domestic and imported IFM and MBBF was performed by DLLME base on novel hydrophobic DES after SPE (SPE−DLLME−DES) followed by HPLC combined with fluorescence detection (HPLC−FL).

In Iran, many studies have been performed on the evaluation of AFM1 in raw milk (Pour et al. 2020), pasteurised and sterilised milk (Ghanem and Orfi 2009; Heshmati and Milani 2010), breast milk (Fakhri et al. 2019) and other dairy products such as cheese, yogurt, buttermilk, etc. (Pour et al. 2020) that in some studies, the AFM1 level is reported to be higher than the standard allowable level. Unlike the above products, there is limited information about AFM1 in IFM and MBBF consumed in Iran (Oveisi et al. 2007). Therefore, continuous monitoring of the quality of these products in terms of AFM1 in Iran as in other countries is necessary. So far, various studies have been conducted on the evaluation of AFM1 in IFM and MBBF in different countries, including Turkey (Baydar et al. 2007), Argentina (Londoño et al. 2013), Portugal (Alvito et al. 2010), Lebanon (Elaridi et al. 2019), China (Li et al. 2018), Jordan (Awaisheh et al. 2019), India (Kanungo & Bhand 2014), Pakistan (Akhtar et al. 2017), Brazil (Ishikawa et al. 2016), Mexico (Quevedo-Garza et al. 2020) and other countries, and in some studies, the level of AFM1 in IFM and MBBF has been reported to be higher than the standard allowable level. The standard of the EU, Switzerland, USA, Australia for the maximum allowable AFM1 in infant formula food (IFF) is 25, 10, 50 and 10 ng/kg, respectively (FDA, CPG 2005; EC 2006). The above standard in Iran is equal to 25 ng/kg (ISIRI 2020). In Iran, the standard announced for AFM1 in IFM/MBBF is adapted from the standard of other countries, especially the United States, and is not a native and specific standard of Iran. Therefore, it seems that performing human health risk assessment related to AFM1 in IFM/MBBF is a more appropriate solution to determine whether IFM/MBBF is safe or unsafe in Iranian markets (according to AFM1), because the amount of consumption, frequency and duration of IFM/MBBF consumption, lifestyle and an average weight of IFM/MBBF consumers in Iran is different from other countries (Sharafi et al. 2022). Other innovations of this study are health risk assessment using various health risk indicators, such as liver cancer risk (LCR), margin of exposure (MOE) and target hazard quotient (THQ), which has not been done in most previous similar studies in Iran.

In terms of the analysis of AFM1 it should be noted, that the selection of DES constituents, obtaining the appropriate molar ratio between

them and the temperature conditions are among the most important parameters that must be considered in the synthesis of DES. In our previous research (Rostami-Javanroudi et al. 2021), we synthesised a new DES and optimised all synthesis conditions and effective parameters. The efficiency of the synthesised DES was investigated in the extraction and pre-concentration of organic compounds. In the present research, the same DES was used as the extraction solvent in DLLME step and the repetition of the synthesis method and the study of the parameters affecting the synthesis were avoided. Only the conditions of SPE and DLLME together with some notable parameters in the combination of SPE–DLLME were investigated.

Materials and methods

Reagents and solutions

Stock solutions of AFM1 were obtained from Sigma-Aldrich (St. Louis, MO). Working solutions were prepared in methanol: water (50:50%) at a final concentration of 100 µg L^{-1} by diluting intermediate solutions (100 mg L^{-1}, 10 mg L^{-1} and 1 mg L^{-1}). HPLC-grade methanol and acetonitrile, phosphate salt (analytical grade), NaCl, ethylene glycol, *n*-butanol, glycerol, *n*-heptanol and *n*-nonanol were acquired from Merck (Darmstadt, Germany). Methyl trioctyl ammonium chloride (MTOAC) (>97%) was obtained from Aladdin Biochemical Co., Ltd. (Shanghai, China). Water used in experiments and the mobile phase was ultra-pure water from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran).

Instrumentation

Chromatographic analyses of AFM1 were performed by HPLC (Knauer-Azura, Berlin, Germany) consisting of an online vacuum degasser, a quaternary pump and a fluorescence detector (RF-20A) coupled to a photochemical post-column reactor for derivatisation of AFM1 with UV-Light (LC Tech, Clearwater, FL). The excitation and emission wavelengths of the fluorescence detector were set to 360 and 440 nm, respectively. A C_{18} column (250 × 4.6 mm ID, 5 µm particle size) analytical column with a pre-column (Knauer, Berlin, Germany) was used for the separation of AFM1 and the column temperature was set at 40 °C. The mobile phase consisted of acetonitrile/water (70:30 v/v) at a flow rate of 1.0 mL min^{-1}.

Sampling and sample preparation

Based on the results of previous studies and using NCSS software, the minimum number of required samples was estimated to be 105, of which 114 samples were considered in this study. Thirty-eight widely consumed brands including DIFM (12 brand), IIFM (8 brand), DMBBF (12 brand) and IMBBF (6 brand) were purchased from different drugstores in Kermanshah city, Iran. From each brand, three samples were selected, so for this study, 114 samples were analysed.

For sample preparation, exact amount of 1.0 g of each sample was placed in a 20-mL test tube, then 10 mL of water at a temperature of about 50 °C was added to it to dissolve completely and obtain a homogeneous solution. After cooling the solution to room temperature, its volume was increased to 20 mL with distilled water. The resulting solution is centrifuged at a speed of 10,000 rpm at 4 °C for 10 min to remove cream and fat. After the fat was removed, the sample was filtered through a glass microfiber filter (Glass Microfiber Filter, GF/A, 1.6 µm core size, Whatman, Maidstone, UK). The final solution was subjected to the SPE−DLLME−DES procedure.

SPE−DLLME−DES procedure

An SPE-100 mg of the Bond Elute PPL sorbent (3 mL, syringe barrel, Varian, Harbor City, CA) cartridge was used for aflatoxin clean-up. The cartridge was conditioned with 2.0 mL of acetonitrile, water and water at pH 2.5, respectively. The sample was loaded at a flow rate of about 4 mL min^{-1} and the SPE cartridge was rinsed with 5 mL of water to remove any matrix interferences. After drying the column by applying gentle vacuum, the AFM1 was eluted slowly from the column by passing 1.0 mL acetonitrile. The acetonitrile was allowed to be in contact with column at least 2 min. The acetonitrile was collected into the 10-mL screw cap glass test tubes and

50 µL of DES were added to the test tube. Then, 5.0 mL distilled water was rapidly injected into a test tube. A cloudy solution, resulting from the dispersion of the fine DES droplets in the aqueous solution, was formed in the test tube. In this step, the AFM1 was extracted into the fine droplets of DES. The mixture was then centrifuged for 5 min at 4000 rpm, until the dispersed fine particles of the DES floated to the top of the test tube. The upper phase was completely transferred to a conical sample cup and 20 µL of this phase was injected into the LC−FL.

Human health risk assessment

Estimated daily intake (EDI)

To assess the human health risk of exposure to AFM1, the daily intake of AFM1 was first calculated using Equation (1).

$$\mathrm{EDI}\ \left(\mathrm{ng/kg\ bw/day}\right) = \frac{C_{AFM1}\left(\frac{ng}{kg}\right) \times IR_{IDPM}\ \left(\mathrm{g/day}\right)}{\mathrm{BW}\ \left(\mathrm{kg}\right)} \quad (1)$$

In this equation, EDI is estimated daily intake (EDI), C is AFM1 concentration and IR is daily intake of IFM, which for Iranian infants less than 6, 7−8 and 9−12 months are equal to 53.3−106.6, 40−80 and 26.7−53.4 g/d, respectively (García-Moraleja et al. 2015). BW is the average body weight (BW) equal to 5.7 ± 0.81, 6.9 ± 0.51 and 9.5 ± 0.98 kg for the above age groups for Iranian infants (Table S1) (García-Moraleja et al. 2015).

Carcinogenic risk assessment

The AFM1-related LCR assessment method is provided by The Joint FA/WHO Expert Committee on Food Additives (JECFA) (Joint 2017). The JECFA has reported the potential for liver cancer exposure at 1 ng AFB1/kg BW/day per 100,000. According to the report, the upper boundaries of AFM1's potential for LCR for people with HBsAg− (hepatitis B surface antigen-negative) and HBsAg+ (positive) are 0.049 and 0.0562 of additional cancer cases per 100,000, respectively. Since the carcinogenic potency of AFM1 is 0.1 of AFB1 even in susceptible species such as rainbow trout and fisher rat, the carcinogenic potency of AFB1 for HBsAg−and HBsAg+ has been reported to be 0.0049 and 0.0562, respectively (Joint 2017; Hooshfar et al. 2020). According to the Center for Disease Control and Prevention of Iran, the prevalence of HBsAg+ in Iran is equal to 1.5%, so the population (Pop) is associated with HBsAg+ and HBsAg− is equal to 0.015 and 0.985, respectively (Hooshfar et al. 2020). Based on the above information, Equations (2) and (3) were used to calculate the carcinogenic potential (cancer potency [CP]) and cancer risk (CR) created by AFM1. Finally, the value of CP was obtained 5.7E-03.

$$\mathrm{CP} = \left(\mathrm{PHBsAg^{+}} \times \mathrm{\%PopHBsAg^{+}}\right) + \left(\mathrm{PHBsAg^{-}} \times \mathrm{\%PopHBsAg^{-}}\right) \quad (2)$$

$$\mathrm{CR} = \mathrm{CP} \times \mathrm{ADI} \quad (3)$$

Margin of exposure

MOE is another way of expressing the health risk posed by exposure to contaminants (EFSA Scientific Committee 2005). A Benchmark Dose (BMD) or a Benchmark Dose Lower Confidence Limit of 10% (BMDL10) can be used to calculate the MOE. BMD is the dose that causes the least but measurable effects, while BMDL10 is the minimum dose that causes cancer incidence of no more than 10% with 95% confidence. In this study, MOE related to AFM1 is calculated by using IDPM by Equation (4) (Hooshfar et al. 2020).

$$\mathrm{MOE} = \frac{570\ \mathrm{ng/kg\ bw/day}}{\mathrm{ADI}\ \left(\mathrm{ng/kg\ bw/day}\right)} \quad (4)$$

In Equation (4), the value of 570 ng/kg BW/d is the reference value equal to the value of AFM1, a two-year study that causes hepatocellular carcinoma in male Fischer rats (Udovicki et al. 2019). If the MOE value is equal to and more than 10,000, it causes fewer health risks and therefore has a lower priority for risk management actions. At the same time, if the MOE is <10,000, this shows that exposure to that contaminant poses potential risks to public health. As a result, risk management measures should be prioritised (Udovicki et al. 2019; Hooshfar et al. 2020).

Hazard quotient (HQ)

In this study, CR and MOE were calculated first, but to be surer of IDPM safety in terms of AFM1, the HQ value was also calculated using Equation (5) (Ishikawa et al. 2016).

$$HQ = \frac{ADI\ (ng/kg\ bw/day)}{RFD\ (ng/kg\ bw/day)} \quad (5)$$

In this equation, ADI and RFD are the average daily intake of AFM1 due to IDPM consumption and the reference dose, respectively. TD50 of AFM1 (10.38 µg/kg BW/d) was used (Kuiper-Goodman 1990). The TD50 is equal to some AFM1, which causes tumours in half of the laboratory animals. To obtain the AFM1-related RFD in humans, the TD50 was then divided by an uncertainty factor of 50,000 (which poses a risk of 1:100,000). Finally, based on the above explanations, at the denominator of Equation (6), the RFD value was considered equal to 0.2 ng/kg BW/day (Hooshfar et al. 2020; Ishikawa et al. 2016) If the value of HQ is more than 1, it is considered an unacceptable risk, and if it is equal or less than 1, it is considered an acceptable risk (Kuiper-Goodman 1990; Ishikawa et al. 2016; Udovicki et al. 2019; Sharafi et al. 2022).

Statistical analyses

IBM SPSS version 23.00 (SPSS Inc., Chicago, IL) was used for statistical analysis. Due to the normality of the data ($p > 0.05$), which was detected by the Komologorov–Smirnov test, the one-way analysis of variance (ANOVA) parametric test was used at a significant level ($\alpha = 0.05$) to compare the mean AFM1 levels between brands and IFM/MBBF.

Results and discussion

In this study, the combination of SPE and DLLME based on a novel hydrophobic DES was designed and employed for the extraction and pre-concentration of AFM1 in domestic and imported IFM/MBBF samples. To obtain good results, the parameters affecting the SPE and DLLME must be tested and optimised.

Optimisation of SPE parameters

When using SPE cartridges, there are several parameters that strongly affect the extraction efficiency. These parameters are: flow rate of the sample solution, breakthrough volume, sample solution pH, salt effect and elution solvent type and volume. In this study, all parameters were examined separately and the optimal conditions were selected. For this purpose, experiments were designed in which the desired parameter was changed and other parameters were kept constant. The results are illustrated in Figure 1. Based on the results shown in Figure 1, the following conditions were chosen as the optimum parameters for the SPE procedure: flow rate of the sample solution, 4 mL min^{-1}; breakthrough volume of 20 mL; sample solution pH of 6.5; elution solvent type and volume of acetonitrile, 1.0 mL; no salt addition.

Optimisation of DLLME parameters

Important parameters in the DLLME method are the type of extraction solvent, the type of dispersion solvent, the volume of the extraction solvent and the volume of the dispersion solvent. In the combination of SPE and DLLME, the cartridge elution solvent during the SPE stage should act as a dispersion solvent at the DLLME stage. Due to the fact that in the SPE stage, acetonitrile with a volume of 1.0 mL was selected as a cartridge elution solvent, as a result, acetonitrile and its volume of 1 mL were used as the type and volume of disperser solvent in the DLLME stage.

As mentioned above, in this study DES was used as the extractant in the DLLME stage. In our previous research (Rostami-Javanroudi et al. 2021), we synthesised a new DES and this DES was used as the extraction solvent for the DLLME stage. For this purpose, five DESs including MTOAC:ethylene glycol, MTOAC:*n*-butanol, MTOAC:glycerol, MTOAC:*n*-heptanol and MTOAC:*n*-nonanol were used as possible extraction solvent. The results for the enrichment factor of the AFM1 showed that MTOAC:*n*-butanol in molar ratio of 1:3 was more effective that other solvents (Figure 2(A)).

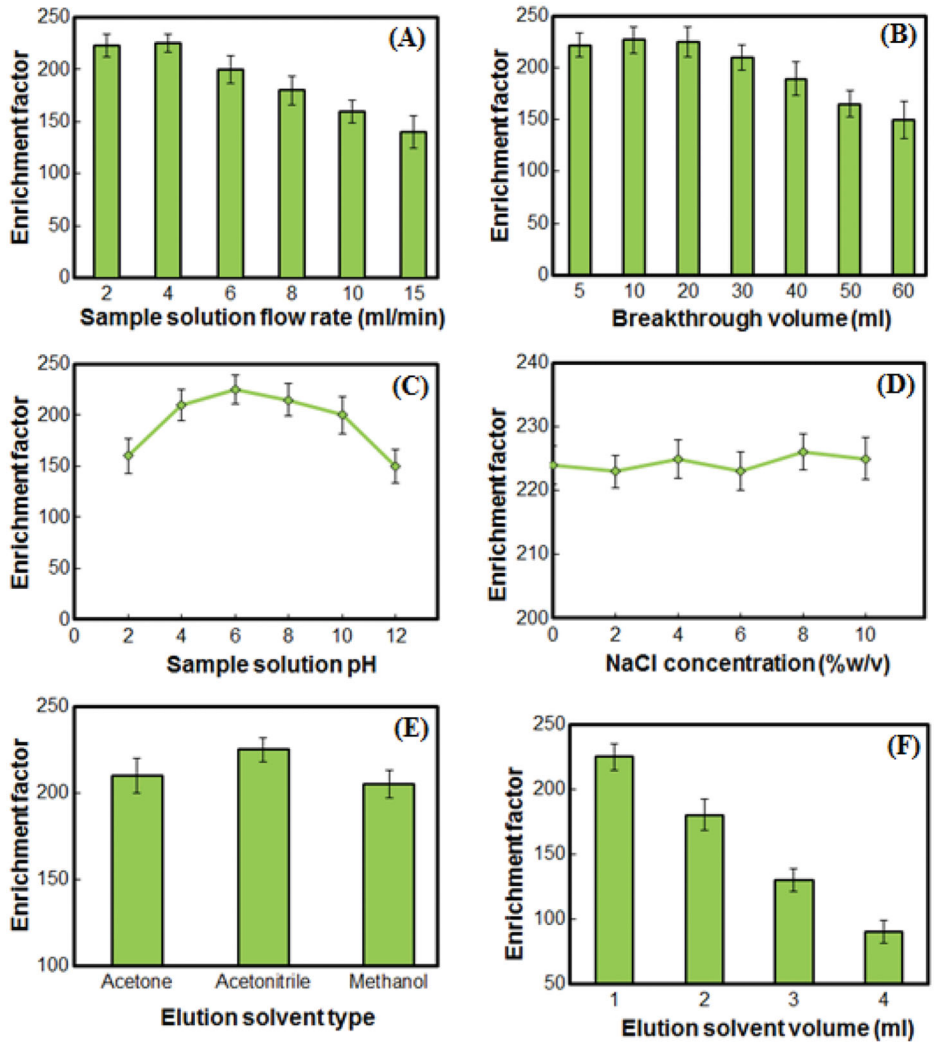

Figure 1. Effect of the sample solution flow rate (A), breakthrough volume (B), sample solution pH (C), NaCl concentration (D), elution solvent type (E) and elution solvent volume (F) on the enrichment factor of the AFM1. All experiments were repeated 3 times and the standard deviation of 3 times was shown as an error bar.

The DES volume is important factor which can directly affected the extraction recovery of AFM1 and subsequently the quantification and detection limit of the method. To examine the effect of this parameter, different volumes of DES including 30, 40, 50, 60, 70 and 80 µL were used. The results in Figure 2(B) show, by increasing the DES volume from 30 to 50 µL, the extraction recovery of AFM1 increased and by further increasing the DES volume, the extraction recovery slightly decreased because of a dilution effect. At volumes less than 30 µL, the volume of the final extraction phase was less than 20 µL, which is not sufficient for injection into the LC and leads to systematic errors. Therefore, 50.0 µL of DES was selected as a compromise in order to obtain higher extraction recovery and lower detection limit.

Analytical figures of merit

Analytical features of the presented method were examined under the most favourable conditions and achieved results are summarised in Table 1. Method validation was done according to the US Food and Drug Administration (FDA) bioanalytical validation guidelines (USFDA 2001). The method was evaluated for accuracy and precision by analysis of quality control (QC) sample at four concentration levels (including 0.05, 0.50, 1.0 and 5.0 µg kg^{-1}) within the calibration range in IFM and MBBF. The prepared samples were

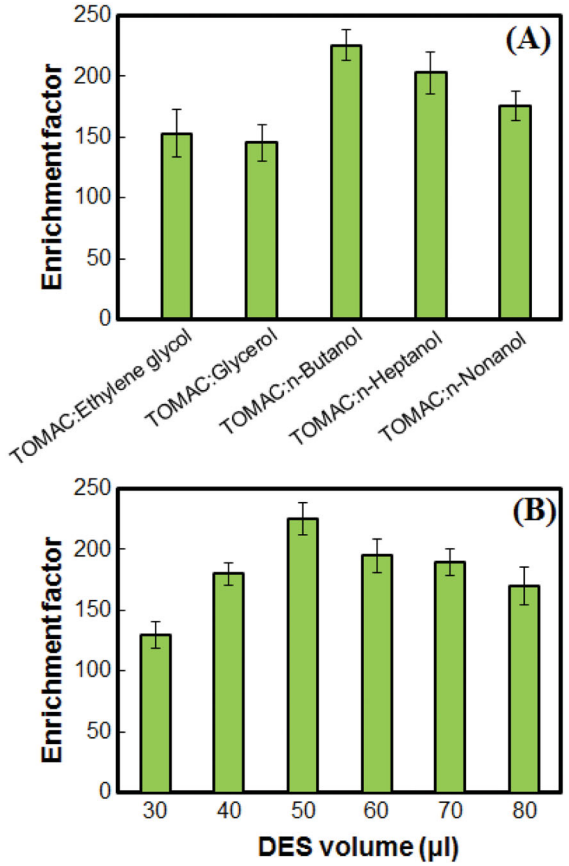

Figure 2. Effect of the DES type (A) and DES volume (B) on the enrichment factor of the AFM1. All experiments were repeated 3 times and the standard deviation of 3 times was shown as an error bar.

Table 1. Analytical characteristics of SPE – DLLME – DES followed by HPLC – FL for determination of AFM1.

Parameter	Analytical feature
Linear range (µg kg^{-1})	0.005–10
RSD% (intra-day, $n = 7$)	2.7
RSD% (inter-day, $n = 7$)	3.8
Accuracy% (intra-day, $n = 7$)	90.4–105.3
Accuracy% (inter-day, n = 7)	92.1–106.3
r^2	0.9988
Limit of detection (µg kg^{-1}) (S/N = 3, $n = 7$)	0.002
Limit of quantification (µg kg^{-1}) (S/N = 10, $n = 7$)	0.005
Extraction recovery (%)	89
Enrichment factor	225

analysed in seven replicates on the same day for intra-day, and the same samples were analysed on three consecutive days, for inter-day. For this purpose, specific quantity of AFM1 was added to the known amount of baby food samples. Then the AFM1 was extracted using proposed procedure and the samples were analysed by optimised LC-FL. The quantity recovered from baby food was estimated using respective regression equations. The accuracy was expressed as percent recovery and precision was depicted as percent relative standard deviation (RSD). RSDs including intra-day and inter-day of method based on seven replicate determinations of AFM1 were 2.7 and 3.8%, respectively. The inter-day and intra-day accuracy ranged from 93.5 to 105.2% and 90.8 to 108.0%, respectively. The correlation coefficient (r^2) of the calibration curve was 0.9988. The linear dynamic ranges (LDRs) of the method were evaluated using standard solutions spiked with various concentrations of AFM1. The LDRs were achieved in the range of 0.005 – 10 µg kg^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were estimated based on S/N = 3 and S/N = 10, equal with 0.002 and 0.005 µg kg^{-1}, respectively. The LLOQ was defined as the lowest concentration in the calibration curve that can be measured with acceptable accuracy and precision (≤20%). The LLOQ for AFM1 was 0.005 µg kg^{-1} with accuracy 95.3%. In addition, the EF was calculated at 225 for AFM1 in IFM and MBBF samples.

Analysis of AFM1 in IFM and MBBF samples

The developed method was applied to determination of AFM1 in 150 samples of domestic and imported IFM and MBBF. The results showed that the lowest and highest AFM1s were 8.5 ± 0.9 and 73.0 ± 5.8 ng/kg for DIFM, 7.6 ± 0.9 and 27.5 ± 2.8 ng/kg for IIFM, 9.5 ± 1.2 and 51.5 ± 3.9 ng/kg for DMBBF, and 11.0 ± 0.9 and 32.6 ± 1.4 ng/kg for IMBBF, respectively (Table 2). Based on the results of statistical analysis, it was found that the mean of AFM1 between three different types of infant foods (including DIFM, IIFM and DMBBF) were significantly different from each other ($p = 0.009$). In addition, based on the results, it was found that the average AFM1 was significantly different between various brands related to each type of evaluated infant food ($p < 0.001$) (Table 2).

According to the results, it was found that the average rate of AFM1 in 41.6% of DIFM samples, 12.5% of IIFM samples, 66.7% of DMBBF samples and 33.3% of IMBBF samples was higher than the EU standard (25 ng/kg) and the national standard of Iran (25 ng/kg) (Commission Regulation (EC) 2006; ISIRI 2020). In order to

Table 2. The comparison of AFM1 concentration in various infant formula milk and milk-based baby food brands.

			Comparison of brands	
Types	Brands	Sample number	Mean ± SD (ng/kg)	*p* Value
DIFM	B1-DIFM	3	13.2 ± 2.4	<0.001
	B2-DIFM	3	28.2 ± 1.7	
	B3-DIFM	3	17.9 ± 1.5	
	B4-DIFM	3	23.1 ± 3.2	
	B5-DIFM	3	8.5 ± 0.9	
	B6-DIFM	3	73.0 ± 5.8	
	B7-DIFM	3	56.6 ± 3.7	
	B8-DIFM	3	14.3 ± 1.1	
	B9-DIFM	3	22.1 ± 3.5	
	B10-DIFM	3	18.5 ± 1.6	
	B11-DIFM	3	44.0 ± 3.5	
	B12-DIFM	3	37.2 ± 2.6	
	Total	36	29.7 ± 19.5	–
IIFM	B1-IIFM	3	11.4 ± 1.5	<0.001
	B2-IIFM	3	8.6 ± 0.9	
	B3-IIFM	3	12.4 ± 1.1	
	B4-IIFM	3	23.1 ± 2.2	
	B5-IIFM	3	27.5 ± 2.8	
	B6-IIFM	3	12.2 ± 1.3	
	B7-IIFM	3	9.3 ± 1.0	
	B8-IIFM	3	7.6 ± 0.9	
	Total	24	14.0 ± 7.3	–
DMBBF	B1- DMBBF	3	51.5 ± 3.9	<0.001
	B2- DMBBF	3	37.6 ± 1.9	
	B3- DMBBF	3	41.0 ± 3.3	
	B4- DMBBF	3	11.3 ± 2.4	
	B5- DMBBF	3	32.8 ± 2.8	
	B6- DMBBF	3	18.4 ± 1.9	
	B7-DMBBF	3	9.5 ± 1.2	
	B8-DMBBF	3	14.6 ± 1.6	
	B9-DMBBF	3	19.3 ± 1.7	
	B10-DMBBF	3	26.7 ± 2.1	
	B11-DMBBF	3	39.5 ± 1.8	
	B12-DMBBF	3	46.3 ± 3.4	
	B12-DMBBF	3	28.5 ± 2.3	
	Total	36	29.0 ± 13.7	–
IMBBF	B1-IMBBF	3	16.4 ± 1.2	<0.001
	B2-IMBBF	3	21.0 ± 1.6	
	B3-IMBBF	3	12.4 ± 1.3	
	B4-IMBBF	3	32.6 ± 1.4	
	B5-IMBBF	3	28.9 ± 1.5	
	B6-IMBBF	3	11.0 ± 0.9	
	Total	18	20.4 ± 8.8	–

DIFM: domestic infant formula milk; IIFM: imported infant formula milk; DMBBF: domestic milk-based baby food; IMBBF: imported milk-based baby food

standardise a contaminant, each country must consider the economic situation, lifestyle, duration and the amount of consumption, and in general, the amount of exposure to that pollutant (Pirsaheb et al. 2021). For example, the consumption of IDPM varies from country to country. Baker et al. (2016) reported that IFM consumption in low-, middle- and high-income countries were 2.9, 16.3 and 32 kg per year, respectively, and this amount for the Turkey, Japan, China, USA, Russia, France, Brazil, Mexico, Iran, India, Nigeria, Pakistan, UK, Indonesia and the Philippines have been reported equal to 4.7, 26.3, 21.7, 45.4, 22.8, 13.6, 7.5, 8.3, 7.0, 0.9, 0.4, 0.1, 65.7, 13.9 and 11.7 kg per year, respectively (Baker et al. 2016). Higher consumption of IFM and MBBF places infants to more exposure and poses a higher potential risk to consuming infants, therefore, citing a specific international standard such as the EU standard (Commission Regulation (EC) 2006) may not provide high reliability and safety for all countries. It seems that the Iranian national standard for AFM1 in IFM and MBBF (25 ng/kg) has been adapted directly from international guidelines without conducting field research in the Iranian neonatal community. Therefore, in such circumstances, it is necessary to conduct a human health risk assessment that was performed in this study. Various studies have been conducted on the studied subject both in Iran and in other countries; the results of this study are consistent with the results of some of them and are not consistent with others (Table 3). While in this study, the mean AFM1 in DIFM, IIFM, DMBBF and IMBBF samples were 29.7 ± 19.5, 14.0 ± 7.3, 29.0 ± 13.7 and 20.4 ± 8.8, respectively, and 100 studied samples for AFM1 were positive (Table 2). The results of this study are completely different from the results of other studies in other countries, the most important reason being the quality and storage conditions of feed lactating cows, which directly affect the production of AFB1 in the milk of these animals (Rastogi et al. 2004; Ghanem and Orfi 2009; Noori et al. 2013). Therefore, there is a linear relationship between AFM1 and AFB1 so that approximately 0.3−6.2% of AFB1 is converted to AFM1 through diet (Oveisi et al. 2007; Alvito et al. 2010; Sharafi et al. 2022).

Health risk assessment of AFM1 in IDPM

Estimated daily intake (EDI) of AFM1

If the mean is considered as one of the independent parameters affecting the EDI, including aflatoxin concentration (C-AFM1), BW and IFM/MBBF (IR) consumption. In that case, the calculated EDI value will be only as a point estimation (PE) of EDI. The results of this study showed that the rate of PE of ADI for age group <6, 7−8 and 9−12 months is equal to 0.42, 0.26 and 0.13 ng/kg BW/d for DIFM consumption and

Table 3. AFM1 levels in IFM from similar previous published studies.

Study area	Positive samples (%)	Number of samples higher than EC standard	Mean ± SD (ng/kg)	References
Iran	3.4	0	21.7 ± 0	Hooshfar et al. (2020)
Iran	64.6	100	328 ± 209	Noori et al. (2013)
Iran	100	80.7	324 ± 3.21	Kamkar (2008)
Iran	96.6	0	7.31 ± 3.91	Oveisi et al. (2007)
Turkey	36.5	0	0.06 ± 0.03	Baydar et al. (2007)
Argentina	100	100	393 ± 240	Londoño et al. (2013)
Brazil	100	100	346 ± 296	Londoño et al. (2013)
Brazil	43	18.8	24 ± 10	Ishikawa et al. (2016)
India	94	94	326 ± 45	Rastogi et al. (2004)
Jordan	100	85	120 ± 33.5	Omar (2016)
Jordan	48.3	48.3	74.2 ± 7.50	Awaisheh et al. (2019)
Lebnan	88.1	31	20.1 ± 1.30	Elaridi et al. (2019)
Turkey	2.9	0	6.10 ± 0	Er et al. (2014)
Mexico	20	20	440 ± 1089	Quevedo-Garza et al. (2020)
Pakistan	53.8	30.8	6.31 ± 10.5	Akhtar et al. (2017)
Portugal	85.7	14.3	12.1 ± 13.4	Alvito et al. (2010)
Syrian	13	0	12 ± 0	Ghanem and Orfi (2009)
Turkey	16.7	0	16 ± 0	Kabak (2012)
Italy	1.1	0	13.5 ± 2.52	Meucci et al. (2010)
Egypt	10	0	5 ± 0	Abd Alla et al. (2000)
Italy	0	0	ND	Juan et al. (2014)

0.20, 0.12 and 0.06 ng/kg BW/d for IIFM consumption, 0.41, 0.25 and 0.12 ng/kg BW/d for DMBBF consumption, and 0.29, 0.18 and 0.09 ng/kg BW/d for IMBBF consumption, respectively (Table S2). AFM1-related EDI levels through IFM use in infants less than 6 months of age in Hooshfar et al. (2020) was 0.074 ng/kg BW/d, which was lower than estimated in this study. Considering that in the two studies mentioned above, the parameters of BW and IR are considered almost the same, so the main reason for the difference in EDI reported in the two studies is related to the difference in the concentration of AFM1. Based on this, the type of brand and location of sampling are analysed on the different quality of IFM/MBBF, and as a result, the concentration of AFM1 can be affected.

Risk characterisation

In this study, AFM1 health risk through consumption of domestic and foreign IFM and MBBF by infants less than one-year-old was evaluated by determining three indicators of LCR, MOE and ADI (EFSA Scientific Committee 2005; Joint 2017; Udovicki et al. 2019; Quevedo-Garza et al. 2020). In this study, the first indicator determined to assess AFM1 health risk due to IFM and MBBF use in Iranian infants less than one-year-old was THQ, which this indicator for infants aged <6, 7−8 and 9−12 months related to DIFM were higher than acceptable in 75, 50 and 16.7% of brands, respectively (THQ = 1). These values were 37.5, 12.5 and 0% for IIFM, respectively. Accordingly, DMBBF was 75, 50 and 0%, respectively, and for IMBBF was 66.7, 33.3 and 0%, respectively (Table 4 and Figure 3).

Based on the above results, the THQ for infants <6 months in most brands was higher than the allowable level (THQ = 1) due to the low weight of infants <6 months, which requires special attention. In brands of IFM and MBBF where the THQ level was lower than the allowable level, it should be noted that Iranian infants may be exposed to AFM1 through other sources (including breast milk, raw milk and pasteurised milk). Therefore, it is necessary to take actions, such as continuous monitoring of IFM and MBBF quality offered in Iranian markets, comprehensive studies on AFM1 levels in other infant food sources, and actions to reduce AFM1 levels in domestic IFM and MBBF factories.

This study results are consistent with the results of some similar studies of the past but are not consistent with others. In Hooshfar et al. (2020), the THQ related to AFM1 of IFM has been reported 0.37. In Brazil, Ishikawa et al. Obtained 1.53, 1.26, 0.64 and 0.42 for 1-week-old, 1, 6 and 12-month-old female infants, respectively, while it was obtained 1.48, 1.18, 0.58 and 0.39, respectively, for male infants (Ishikawa

Table 4. The target hazard quotient (THQ) of AFM1 *via* consumption of various infant formula milk and milk-based baby food brands.

		THQ		
Types	Brands	Age ≤ 6 months	7 ≤ Age ≤ 8 months	9 ≤ Age ≤ 12 months
DIFM	B1-DIFM	0.93	0.57	0.28
75%	B2-DIFM	1.98	1.23	0.59
	B3-DIFM	1.26	0.78	0.38
	B4-DIFM	1.62	1.00	0.49
	B5-DIFM	0.60	0.37	0.18
	B6-DIFM	5.12	3.17	1.54
	B7-DIFM	3.97	2.46	1.19
	B8-DIFM	1.00	0.62	0.30
	B9-DIFM	1.55	0.96	0.47
	B10-DIFM	1.30	0.80	0.39
	B11-DIFM	3.09	1.91	0.93
	B12-DIFM	2.61	1.62	0.78
	Total	2.09	1.29	0.63
IIFM	B1-IIFM	1.37	0.85	0.41
	B2-IIFM	0.80	0.50	0.24
	B3-IIFM	0.60	0.37	0.18
	B4-IIFM	0.87	0.54	0.26
	B5-IIFM	1.62	1.00	0.49
	B6-IIFM	1.93	1.20	0.58
	B7-IIFM	0.86	0.53	0.26
	B8-IIFM	0.65	0.40	0.20
	Total	0.53	0.33	0.16
DMBBF	B1-DMBBF	0.98	0.61	0.30
	B2-DMBBF	0.51	0.32	0.15
	B3-DMBBF	3.61	2.24	1.08
	B4-DMBBF	2.64	1.63	0.79
	B5-DMBBF	2.88	1.78	0.86
	B6-DMBBF	0.79	0.49	0.24
	B7-DMBBF	2.30	1.43	0.69
	B8-DMBBF	1.29	0.80	0.39
	B9-DMBBF	0.67	0.41	0.20
	B10-DMBBF	1.02	0.63	0.31
	B11-DMBBF	1.35	0.84	0.41
	B12-DMBBF	1.87	1.16	0.56
	B12-DMBBF	2.77	1.72	0.83
	Total	3.25	2.01	0.97
IMBBF	B1-IMBBF	2.00	1.24	0.60
	B2-IMBBF	2.04	1.26	0.61
	B3-IMBBF	0.96	0.60	0.29
	B4-IMBBF	1.15	0.71	0.35
	B5-IMBBF	1.47	0.91	0.44
	B6-IMBBF	0.87	0.54	0.26
	Total	2.29	1.42	0.69

DIFM: domestic infant formula milk; IIFM: imported infant formula milk; DMBBF: domestic milk-based baby food; IMBBF: imported milk-based baby food.

et al. 2016). A study by Awaisheh et al, In Jordan showed that for 6 and 12-month-old female infants, the values obtained were estimated to be 7.86 and 7.75, respectively, which were high values (Awaisheh et al. 2019). In other studies, including Spain, Argentina and Thailand, it was estimated in the range of 0.8−18.5 (Cano-Sancho et al. 2010; Alonso et al. 2010; Ruangwises et al. 2011). Differences in the results of previous studies with this study are related to the difference in EDI, which is due to differences in neonatal weight, AFM1 concentration, quality and daily consumption of IDPM (Oveisi et al. 2007; Ishikawa et al. 2016; Awaisheh et al. 2019; Hooshfar et al. 2020).

The second indicator set to assess AFM1 health risk due to IFM and MBBF use in Iranian infants less than one-year-old was LCR. According to the results, the LCR level of AFM1 through consumption of all IFM/MBBF brands by all three age groups of infants less than one year is in the range of 1.7E-04 to 5.8E-03 (in terms of additional cancer cases/year/10^5 Pop) (Table 5 and Figure 3). Based on the results, it can be concluded that LCR due to receiving AFM1 through IDPM consumption by Iranian

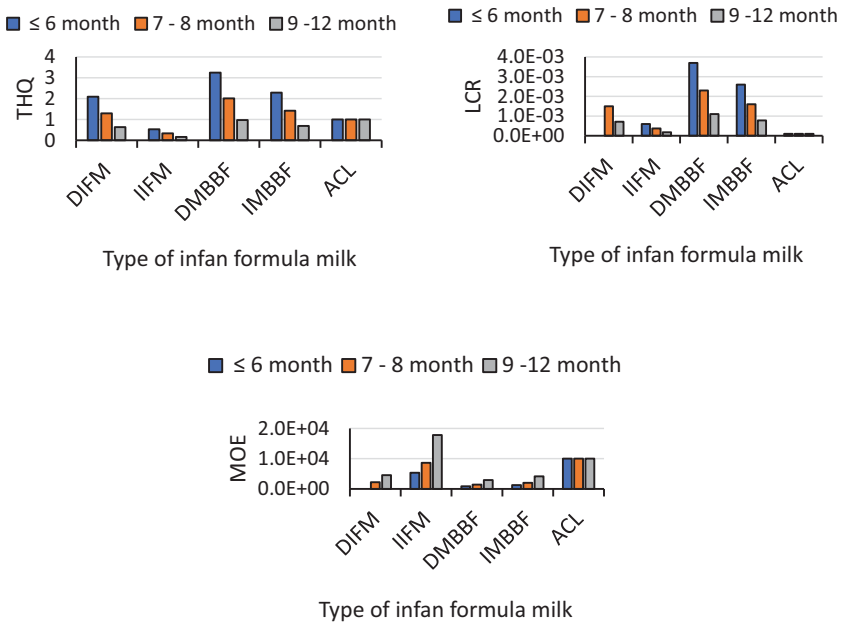

Figure 3. The comparison of THQ, LCR and MOE related to AFM1 in various infant formula milk between different age groups. DIFM: domestic infant formula milk; IIFM: imported infant formula milk; DMBBF: domestic milk-based baby food; IMBBF: imported milk-based baby food; THQ: target hazard quotient; LCR: liver cancer risk; MOE: margin of exposure.

infants less than one-year-old in some of the studied brands, especially for infants less than 6 years old is a worrying health risk. However, in some other cases, it cannot be considered as a significant risk. Similar to this study, Hooshfar et al. (2020) estimated LCR for AFM1 obtained through the use of IDPM by infants less than 6 months of age equal to 1.0E-04 additional cancer cases/year/10^5 Pop. In Pardakhti and Maleki (2019), it was found that the LCR of AFM1 through milk consumption for healthy persons in Tehran, Mashhad, Babol, Isfahan, Kermanshah, Miandoab, Hamedan and Urmia were equal to 5.7E-05, 6.3E-05, 1.2E-04, 2.8E-04, 6.9E-04, 3.3E-03, 6.9E-06, 3.6E-06 and 2.1E-02 (in terms of additional cancer cases/year/10^5 Pop), respectively. For HB-infected persons, these values were obtained 1.7E-03, 1.9E-03, 3.5E-03, 8.5E-03, 2.1E-03, 9.9E-02, 2.1E-04, 1.1E-03 and 6.3E-03 (in terms of additional cancer cases/year/10^5 Pop), respectively. Based on the results, it can be said that due to precautions and control actions in recent years in Iran, the carcinogenicity risk of AFM1 through consumption of milk and milk products, especially IFM and MBBF, is low and is not considered a health concern.

MOE was the third indicator set to assess the health risk associated with AFM1 of IFM/MBBF in this study. If the MOE value is equal to and more than 10,000, it causes fewer health risks and therefore has a lower priority for risk management actions, while if the MOE is less than 10,000, it indicates that exposure to that pollutant has high health risks to society and should be considered as a higher priority for risk management actions (Hooshfar et al. 2020; Sharafi et al. 2022). This indicator was obtained equal to less than 10,000 for all brands of IFM and MBBF types in both age groups <6 and $7-8$ months, which is considered as a health concern for these two age groups while for the age group of $9-12$ months for DIFM, IIFM, DMBBF and IMBBF, the percentage of brands with MOE less than 10,000 was 83.3, 25, 75 and 83.3%, respectively (Table 6 and Figure 3), which these cases are considered as concerns in terms of health risk, and it is necessary to take risk management actions in this regard (Table 6 and Figure 3). Hooshfar et al. (2020) obtained a PE of MOE associated with AFM1 of less than 10,000 (exactly 7671.6) through the use of DIFM for infants less than 6 months of age. Similarly, in this study, the

Table 5. The liver cancer risk (LCR) of AFM1 *via* consumption of various infant formula milk and milk-based baby food brands.

		LCR		
Types	Brands	Age ≤ 6 months	7 ≤ Age ≤ 8 months	9 ≤ Age ≤ 12 months
DIFM	B1-DIFM	1.1E-03	6.5E-04	3.2E-04
	B2-DIFM	2.2E-03	1.4E-03	6.7E-04
	B3-DIFM	1.4E-03	8.8E-04	4.3E-04
	B4-DIFM	1.8E-03	1.1E-03	5.5E-04
	B5-DIFM	6.8E-04	4.2E-04	2.0E-04
	B6-DIFM	5.8E-03	3.6E-03	1.7E-03
	B7-DIFM	4.5E-03	2.8E-03	1.4E-03
	B8-DIFM	1.1E-03	7.0E-04	3.4E-04
	B9-DIFM	1.8E-03	1.1E-03	5.3E-04
	B10-DIFM	1.5E-03	9.1E-04	4.4E-04
	B11-DIFM	3.5E-03	2.2E-03	1.1E-03
	B12-DIFM	3.0E-03	1.8E-03	8.9E-04
	Total	2.4E-03	1.5E-03	7.1E-04
IIFM	B1-IIFM	1.6E-03	9.6E-04	4.7E-04
	B2-IIFM	9.1E-04	5.6E-04	2.7E-04
	B3-IIFM	6.8E-04	4.2E-04	2.1E-04
	B4-IIFM	9.9E-04	6.1E-04	3.0E-04
	B5-IIFM	1.8E-03	1.1E-03	5.5E-04
	B6-IIFM	2.2E-03	1.4E-03	6.6E-04
	B7-IIFM	9.7E-04	6.0E-04	2.9E-04
	B8-IIFM	7.4E-04	4.6E-04	2.2E-04
	Total	6.0E-04	3.7E-04	1.8E-04
DMBBF	B1-DMBBF	1.1E-03	6.9E-04	3.3E-04
	B2-DMBBF	5.8E-04	3.6E-04	1.7E-04
	B3-DMBBF	4.1E-03	2.5E-03	1.2E-03
	B4-DMBBF	3.0E-03	1.9E-03	9.0E-04
	B5-DMBBF	3.3E-03	2.0E-03	9.8E-04
	B6-DMBBF	9.0E-04	5.6E-04	2.7E-04
	B7-DMBBF	2.6E-03	1.6E-03	7.8E-04
	B8-DMBBF	1.5E-03	9.1E-04	4.4E-04
	B9-DMBBF	7.6E-04	4.7E-04	2.3E-04
	B10-DMBBF	1.2E-03	7.2E-04	3.5E-04
	B11-DMBBF	1.5E-03	9.5E-04	4.6E-04
	B12-DMBBF	2.1E-03	1.3E-03	6.4E-04
	B12-DMBBF	3.1E-03	1.9E-03	9.4E-04
	Total	3.7E-03	2.3E-03	1.1E-03
IMBBF	B1-IMBBF	2.3E-03	1.4E-03	6.8E-04
	B2-IMBBF	2.3E-03	1.4E-03	6.9E-04
	B3-IMBBF	1.1E-03	6.8E-04	3.3E-04
	B4-IMBBF	1.3E-03	8.1E-04	3.9E-04
	B5-IMBBF	1.7E-03	1.0E-03	5.0E-04
	B6-IMBBF	9.9E-04	6.1E-04	3.0E-04
	Total	2.6E-03	1.6E-03	7.8E-04

DIFM: domestic infant formula milk, IIFM: Imported infant formula milk, DMBBF: Domestic milk-based baby food, IMBBF: Imported milk-based baby food.

value in most cases was less than 10,000 (exactly equal to 4419.9).

Conclusions

In this work, the combination of SPE and DLLME – DES was successfully used for the extraction and pre-concentration of AFM1 in IFM and MBBF prior to analysis by LC–FL. As compared with the other conventional sample-preparation methods, this method offered numerous advantages, such as environmentally friendly, safe, simple, ease of operation, high enrichment factor and low detection limit. Based on the results, it was found that the average rate of AFM1 in 41.6% of DIFM samples, 12.5% of IIFM samples, 66.7% of DMBBF samples and 33.3% of IMBBF samples was higher than the EU standard (25 ng/kg) and the national standard of Iran (25 ng/kg). In addition, most infants less than one-year-old are exposed to higher than allowable values for health risk-related factors including LCR and MOE. Based on the results, it can be concluded that the quality of IFM and MBBF consumed in Iran in terms of AFM1 is poor. Therefore, exposure to this contaminant has high health risks for infants,

Table 6. The margin of exposure (MOE) of AFM1 *via* consumption of various infant formula milk and milk-based baby food brands.

		MOE		
Types	Brands	Age ≤ 6 months	7 ≤ Age ≤ 8 months	9 ≤ Age ≤ 12 months
DIFM	B1-DIFM	3076.7	4965.9	10255.7
	B2-DIFM	1440.2	2324.5	4800.5
	B3-DIFM	2268.9	3662.0	7562.8
	B4-DIFM	1758.1	2837.7	5860.4
	B5-DIFM	4777.9	7711.8	15926.5
	B6-DIFM	556.3	897.9	1854.5
	B7-DIFM	717.5	1158.1	2391.8
	B8-DIFM	2840.0	4583.9	9466.8
	B9-DIFM	1837.7	2966.1	6125.6
	B10-DIFM	2195.3	3543.2	7317.6
	B11-DIFM	923.0	1489.8	3076.7
	B12-DIFM	1091.7	1762.1	3639.1
	Total	1366.7	2205.8	4555.5
IIFM	B1-IIFM	2081.8	3360.1	6939.3
	B2-IIFM	3562.5	5750.0	11875.0
	B3-IIFM	4722.4	7622.1	15741.3
	B4-IIFM	3275.2	5286.3	10917.3
	B5-IIFM	1758.1	2837.7	5860.4
	B6-IIFM	1476.8	2383.6	4922.7
	B7-IIFM	3328.9	5373.0	11096.3
	B8-IIFM	4366.9	7048.4	14556.5
	Total	5343.8	8625.0	17812.5
DMBBF	B1-DMBBF	2898.3	4678.0	9661.0
	B2-DMBBF	5587.4	9018.2	18624.6
	B3-DMBBF	788.6	1272.8	2628.6
	B4-DMBBF	1080.1	1743.4	3600.4
	B5-DMBBF	990.5	1598.8	3301.8
	B6-DMBBF	3594.0	5800.9	11980.1
	B7-DMBBF	1238.2	1998.5	4127.3
	B8-DMBBF	2207.2	3562.5	7357.3
	B9-DMBBF	4275.0	6900.0	14250.0
	B10-DMBBF	2781.7	4489.7	9272.3
	B11-DMBBF	2104.3	3396.4	7014.2
	B12-DMBBF	1521.1	2455.1	5070.2
	B12-DMBBF	1028.2	1659.5	3427.2
	Total	877.2	1415.8	2923.9
IMBBF	B1-IMBBF	1425.0	2300.0	4750.0
	B2-IMBBF	1400.4	2260.3	4668.1
	B3-IMBBF	2958.6	4775.4	9862.1
	B4-IMBBF	2476.4	3997.0	8254.6
	B5-IMBBF	1933.9	3121.4	6446.4
	B6-IMBBF	3275.2	5286.3	10917.3
	Total	1245.8	2010.7	4152.6

DIFM: domestic infant formula milk; IIFM: imported infant formula milk; DMBBF: domestic milk-based baby.

and this issue should be given special attention for future risk management actions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Abd Alla A, Neamat-Allah A, Aly SE. 2000. Situation of mycotoxins in milk, dairy products and human milk in Egypt. Mycotoxin Res. 16(2):91–100.

Akhtar S, Shahzad MA, Yoo SH, Ismail A, Hameed A, Ismail T, Riaz M. 2017. Determination of aflatoxin M1 and heavy metals in infant formula milk brands available in Pakistani markets. Korean J Food Sci Anim Resour. 37(1):79–86. doi:10.5851/kosfa.2017.37.1.79

Alonso V, Monge M, Larriestra A, Dalcero A, Cavaglieri L, Chiacchiera S. 2010. Naturally occurring aflatoxin M1 in raw milk from farm cooling tanks in Argentina. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 27(3):373–379. doi:10.1080/19440040903403362

Alvito PC, Sizoo EA, Almeida CM, Van Egmond HP. 2010. Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal. Food Anal Methods. 3(1):22–30. doi:10.1007/s12161-008-9064-x

Awaisheh SS, Rahahleh RJ, Algroom RM, Al-Bakheit AA, Al-Khazaleh JF, Al-Dababseh BA. 2019. Contamination level and exposure assessment to Aflatoxin M1 in Jordanian infant milk formulas. Ital J Food Saf. 8:8263.

Baker P, Smith J, Salmon L, Friel S, Kent G, Iellamo A, Dadhich JP, Renfrew MJ. 2016. Global trends and patterns of commercial milk-based formula sales: is an unprecedented infant and young child feeding transition underway. Public Health Nutr. 19(14):2540–2550. doi:10.1017/S1368980016001117

Bakirci I. 2001. A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. Food Control. 12(1):47–51. doi:10.1016/S0956-7135(00)00020-7

Baydar T, Erkekoglu P, Sipahi H, Sahin G. 2007. Aflatoxin B1, M1 and ochratoxin a levels in infant formulae and baby foods marketed in Ankara, Turkey. J Food Drug Anal. 15:89–92.

Cano-Sancho G, Marin S, Ramos AJ, Peris-Vicente J, Sanchis V. 2010. Occurrence of aflatoxin M1 and exposure assessment in Catalonia (Spain). Rev Iberoam Micol. 27(3):130–135. doi:10.1016/j.riam.2010.05.003

Commission Regulation (EC). 2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs No 401/2006. Official Journal of the European Union (OJEU). [accessed 23 February 2006]. https://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32006R0401&from=EN/.

European Food Safety Authority (EFSA) Scientific Committee. 2005. Opinion of the scientific committee on a request from EFSA related to A harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA J. 282:1–31.

Elaridi J, Dimassi H, Hassan H. 2019. Aflatoxin M1 and ochratoxin A in baby formulae marketed in Lebanon: occurrence and safety evaluation. Food Control. 106:106680. doi:10.1016/j.foodcont.2019.06.006

El-Tras WF, El-Kady NN, Tayel AA. 2011. Infants exposure to aflatoxin M1 as a novel foodborne zoonosis. Food Chem Toxicol. 49(11):2816–2819. doi:10.1016/j.fct.2011.08.008

Er B, Demirhan B, Yentür G. 2014. Investigation of aflatoxin M1 levels in infant follow-on milks and infant formulas sold in the markets of Ankara, Turkey. J Dairy Sci. 97(6):3328–3331.

Fakhri Y, Ghorbani R, Taghavi M, Keramati H, Amanidaz N, Moradi B, Nazari SH, Shariatifar N, Khaneghah AM. 2019. Concentration and prevalence of aflatoxin M1 in human breast milk in Iran: systematic review, meta-analysis, and carcinogenic risk assessment: a review. J Food Prot. 82(5):785–795. doi:10.4315/0362-028X.JFP-18-367

Food and Drug Administration (FDA), Compliance Policy Guide (CPG). 2005. CPG Sec. 527.400 whole milk, low fat milk, skim milk-aflatoxin M1. FDA. [accessed 20 October 2019]. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-527400-wholemilk-lowfat-milk-skim-milk-aflatoxin-m1/.

García-Moraleja A, Font G, Mañes J, Ferrer E. 2015. Analysis of mycotoxins in coffee and risk assessment in Spanish adolescents and adults. Food Chem Toxicol. 86:225–233. doi:10.1016/j.fct.2015.10.014

Ghaffarian Bahraman A, Mohammadi S, Jafari A, Ghani-Dehkordid J, Arabnezhad MR, Rahmdel S, Hosseini Teshnizi S. 2020. Occurrence of aflatoxin M1 in milks of five animal species in Iran: a systematic review and meta-analysis. Food Rev Int. 36(7):692–712. doi:10.1080/87559129.2019.1669164

Ghanem I, Orfi M. 2009. Aflatoxin M1 in raw, pasteurized and powdered milk available in the Syrian market. Food Control. 20(6):603–605. doi:10.1016/j.foodcont.2008.08.018

Guo LY, Zheng N, Zhang YD, Du RH, Zheng BQ, Wang JQ. 2016. A survey of seasonal variations of aflatoxin M1 in raw milk in Tangshan region of China during 2012–2014. Food Control. 69:30–35. doi:10.1016/j.foodcont.2016.03.017

Heshmati A, Milani JM. 2010. Contamination of UHT milk by aflatoxin M1 in Iran. Food Control. 21(1):19–22. doi:10.1016/j.foodcont.2009.03.013

Hooshfar S, Khosrokhavar R, Yazdanpanah H, Eslamizad S, Kobarfard F, Nazari F, Kokaraki V, Kokkinakis M, Goumenou M, Tsitsimpikou C, et al. 2020. Health risk assessment of aflatoxin M1 in infant formula milk in IR Iran. Food Chem Toxicol. 142:111455. doi:10.1016/j.fct.2020.111455

Ishikawa AT, Takabayashi-Yamashita CR, Ono E, Bagatin AK, Rigobello FF, Kawamura O, Hirooka EY, Itano EN. 2016. Exposure assessment of infants to aflatoxin M1 through consumption of breast milk and infant powdered milk in Brazil. Toxins. 8(9):246. doi:10.3390/toxins8090246

The Institute of Standards and Industrial Research of Iran (ISIRI). 2020. Food and feed- maximum tolerated level of mycotoxins. ISIRI.No. 5925. p. 11. http://standard.isiri.gov.ir/StandardView.aspx?Id=53664.

The Institute of Standards and Industrial Research of Iran (ISIRI). 2002. Maximum tolerated limits of mycotoxins in foods and feeds. ISIRI. No. A1-5925. p. 1. http://standard.isiri.gov.ir/StandardView.aspx?Id=12991.

Joint Food and Agriculture Organization of the United Nations (FAO). 2017. World Health Organization, WHO Expert Committee on Food Additives. Evaluation of certain contaminants in food: eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland: World Health Organization.

Juan C, Raiola A, Mañes J, Ritieni A. 2014. Presence of mycotoxin in commercial infant formulas and baby foods from Italian market. Food Control. 39:227–236. doi:10.1016/j.foodcont.2013.10.036

Kabak B. 2012. Aflatoxin M1 and ochratoxin A in baby formulae in Turkey: occurrence and safety evaluation. Food Control. 26(1):182–187. doi:10.1016/j.foodcont.2012.01.032

Kamkar A. 2008. Detection of aflatoxin M1 in powdered milk samples by ELISA. Pajouhesh Sazandegi. 21:174–180.
Kanungo L, Bhand S. 2014. A survey of Aflatoxin M1 in some commercial milk samples and infant formula milk samples in Goa, India. Food Agric Immunol. 25(4): 467–476. doi:10.1080/09540105.2013.837031
Khaneghahi Abyaneh H, Bahonar A, Noori N, Yazdanpanah H, Shojaee AliAbadi MH. 2020. Shojaee AliAbadi MH. 2020. The overall and variations of Aflatoxin M1 contamination of milk in Iran: a systematic review and meta-analysis study. Food Chem. 310:125848. doi:10.1016/j.foodchem.2019.125848
Kuiper-Goodman T. 1990. Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin, and zearalenone. Can J Physiol Pharmacol. 68(7):1017–1024. – doi:10.1139/y90-155
Li S, Min L, Wang G, Li D, Zheng N, Wang J. 2018. Occurrence of aflatoxin M1 in raw milk from manufacturers of infant milk powder in China. Int J Environ Res Public Health. 15(5):879. doi:10.3390/ijerph15050879
Londoño VA, Boasso AC, de Paula MC, Garcia LP, Scussel VM, Resnik S, Pacín A. 2013. Aflatoxin M1 survey on randomly collected milk powder commercialized in Argentina and Brazil. Food Control. 34(2):752–755. doi: 10.1016/j.foodcont.2013.06.030
Maki CR, Haney S, Wang M, Ward SH, Bailey RH. 2017. Calcium montmorillonite clay for the reduction of atoxin residues in milk and dairy products. Dairy and Vet Sci J. 2(3):555587.
Maki CR, Haney S, Wang M, Ward SH, Rude BJ, Bailey RH, Harvey RB, Phillips TD. 2017. Calcium montmorillonite clay for the reduction of aflatoxin residues in milk and dairy products. J Dairy Vet Sci. 2(3):555587.
Meucci V, Razzuoli E, Soldani G, Massart F. 2010. Mycotoxin detection in infant formula milks in Italy. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 27(1):64–71. doi:10.1080/02652030903207201
Noori N, Karim G, Raeesian M, Khaneghahi AH, Bahonar A, Akhondzadeh BA, Ghadami F. 2013. Antibiotic residues and aflatoxin M1 contamination in milk powder used in Tehran dairy factories. Iran. Iran J Vet Med. 7: 221–226.
Omar SS. 2016. Aflatoxin M1 levels in raw milk, pasteurised milk and infant formula. Ital J Food Saf. 5:158–160.
Oveisi MR, Jannat B, Sadeghi N, Hajimahmoodi M, Nikzad A. 2007. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. Food Control. 18(10): 1216–1218. doi:10.1016/j.foodcont.2006.07.021
Pardakhti A, Maleki S. 2019. Risk assessment of Aflatoxin M1 contamination of milk in Iran. Int J Environ Res. 13(2):265–271. doi:10.1007/s41742-019-00172-1
Pirsaheb M, Fattahi N, Rahimi R, Sharafi K, Ghaffari HR. 2017. Evaluation of abamectin, diazinon and chlorpyrifos pesticide residues in apple product of Mahabad region gardens: Iran in 2014. Food Chem. 231:148–155. doi:10.1016/j.foodchem.2017.03.120
Pirsaheb M, Hadei M, Sharafi K. 2021. Human health risk assessment by Monte Carlo simulation method for heavy metals of commonly consumed cereals in Iran-Uncertainty and sensitivity analysis. J Food Compost Anal. 96:103697. doi:10.1016/j.jfca.2020.103697
Pour SH, Mahmoudi S, Masoumi S, Rezaie S, Barac A, Ranjbaran M, Oliya S, Mehravar F, Sasani E, Noorbakhsh F, et al. 2020. Aflatoxin M1 contamination level in Iranian milk and dairy products: a systematic review and meta-analysis. World Mycotoxin J. 13(1):67–82. doi:10.3920/WMJ2019.2485
Quevedo-Garza PA, Amador-Espejo GG, Salas-García R, Ramos-Peña EG, Trujillo AJ. 2020. Aflatoxin M1 determination in infant formulae distributed in Monterrey, Mexico. Toxins. 12(2):100. doi:10.3390/toxins12020100
Rastogi S, Dwivedi PD, Khanna SK, Das M. 2004. Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. Food Control. 15(4):287–290. doi:10.1016/S0956-7135(03)00078-1
Rostami-Javanroudi S, Moradi M, Sharafi K, Fattahi N. 2021. Novel hydrophobic deep eutectic solvent for vortex-assisted liquid phase microextraction of common acaricides in fruit juice followed by HPLCUV determination. RSC Adv. 11(48):30102–30108. doi:10.1039/d1ra04781g
Ruangwises N, Saipan P, Ruangwises S. 2011. Estimated daily intake of AFM1 in Thailand. In: Guevara-Gonzalez R, editor. Aflatoxins– biochemistry and molecular biology. Rijeka, Croatia: In Tech; p. 439–446.
Scaglioni PT, Becker-Algeri T, Drunkler D, Badiale-Furlong E. 2014. Aflatoxin B1 and M1 in milk. Anal Chim Acta. 829:68–74. doi:10.1016/j.aca.2014.04.036
Sharafi K, Karami Matin B, Omer AK, Mansouri B, Soleimani H, Fattahi N, Sharafi H, Kiani A. 2022. A worldwide systematic literature review for aflatoxin M1 in infant formula milk: human health risk assessment by Monte Carlo simulation. Food Control. 134:108681. doi: 10.1016/j.foodcont.2021.108681
Shuib NS, Saad B. 2022. In-syringe dispersive micro-solid phase extraction method for the HPLC-fluorescence determination of aflatoxins in milk. Food Control. 132: 108510. doi:10.1016/j.foodcont.2021.108510
Tola M, Kebede B. 2016. Occurrence, importance and control of mycotoxins: a review. Cogent Food Agric. 2:1–12.
Tsakiris IN, Tzatzarakis MN, Alegakis AK, Vlachou MI, Renieri EA, Tsatsakis AM. 2013. Risk assessment scenarios of children's exposure to aflatoxin M1 residues in different milk types from the Greek market. Food Chem Toxicol. 56:261–265. doi:10.1016/j.fct.2013.02.024
Udovicki B, Djekic I, Kalogianni EP, Rajkovic A. 2019. Exposure assessment and risk characterization of Aflatoxin M1 intake through consumption of milk and yoghurt by student population in Serbia and Greece. Toxins. 11(4):205. doi:10.3390/toxins11040205
US Food and Drug Administration (USFDA). 2001. Guidance for industry: bioanalytical method validation.