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Determination of aflatoxin M1 and ochraoxin A in pasteurized milk and evaluation the effect of microwave irradiations on their reduction

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ABSTRACT

Due to the extensive use of dairy-products like pasteurized milk, these products should be free of any contaminants such as drug residues, pesticides, mycotoxins, and etc. Mycotoxins (such as aflatoxins, ochratoxin A, and etc.) are a group of toxic and pollutant substances in foodstuffs, animal and poultry feed that are naturally produced from certain types of fungi. The presence of mycotoxins in milk is very dangerous for the health of the consumer. Therefore, the development of fast, efficient and easy methods for investigating the presence of mycotoxins such as aflatoxin M_1 and ochratoxin A in foodstuffs is of great importance. In this research, an efficient analytical method based on the combination of dispersive solid phase extraction and dispersive liquid-liquid microextraction has been proposed for the extraction of aflatoxin M_1 and ochratoxin A from pasteurized milk samples and analyzing them with high-performance liquid chromatography equipped with a fluorescence detector. Under the optimum condition, limits of detections and quantifications within the ranges of 0.23–0.29 and 0.77–0.97 ng L^{-1} were obtained, respectively. Enrichment factors and extraction recoveries has been obtained in the ranges of 232–249 and 83–89%, respectively. Aflatoxin M_1 (in 70% of the examined milk samples) and ochratoxin A (in 10% of the examined milk samples) were found in a number of samples. Also, according to the obtained results, microwave radiation can reduce the amount of aflatoxin M_1 (in the range of 27.6 to 33.5%) and ochratoxin A (in the range of 17.3 to 20.8%).

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1- Introduction

Nowadays, the onset and spread of different kinds of cancer are caused by various factors such as food safety and seriously threaten the health of human communities. Milk and dairy products have effective role in food regimen of the people all around the world because of their nutrients. Considering this, these products must be free of any contaminants like pesticides, mycotoxins, heavy metals and etc. (Aytenfsu et al.,2016). Mycotoxins are very dangerous and well known contaminants. These compounds are produced naturally by certain kinds of fungi in the process of production, maintenance and storing the foodstuff in warm and humid conditions (Binder, 2007). Among the identified mycotoxins, Aflatoxins and Ochratoxin A are notable examples (Chan et al., 2004). Mycotoxins can enter the human body directly (through consuming contaminated food) or indirectly (through consumption of animal products exposed to mycotoxins like milk). These compounds are carcinogenic and do not eliminate during the process of food production, freezing or cooking. Nowadays more than 17 kinds of Aflatoxins are known in the nature that four main kinds of them include G1, G2, B1, and B2 (Herzallah, 2009). Aflatoxin B1 and its hydroxylated metabolite (Aflatoxin M1) are the most toxic Aflatoxins (Afshar et al., 2020). Ochratoxin A is the other toxic mycotoxin that was extracted and purified from Aspergillus Ochrasius strain for the first time. Considering these points, surveillance of the quantity of mycotoxins in the food samples like milk is very important in notifying the nutrition and health authorities and public community.

The most important challenge for improvement of food and dairy industries is to reduce or eliminate the food contaminants. Up to now to reach this goal different procedures like gamma radiation, UV radiation, microwave radiation, heating, oxidation and compost have been used (Rahmani et al., 2015; Nascimento and Azevedo, 2013). These methods can cause blockage of transmission and reduction of their availability to target tissues and their elimination through alteration and destruction of molecular structure of contaminants.

In most cases with regard to high sensitivity, high performance liquid chromatography is used as a tool for analyzing different kinds of mycotoxins (Owusu Kyei-Baffour et al., 2021). But unfortunately, direct analysis of mycotoxins without the use of preparatory methods, because of the complexity of matrices of samples and low concentration values is not possible (Turner et al., 2009). Therefore, developing and using efficient preparatory methods before analyzing the samples is a great importance. Up until now different methods are used for preparing the samples including liquid-liquid extraction, solid phase extraction, dispersive solid phase extraction and dispersive liquid- liquid micro extraction.

Dispersive solid phase extraction is one of the most employed preparatory methods of samples in which the adsorbing particles directly interact with the sample solution. Depending on their acting group, the adsorbing particle can absorb the present compounds in the sample texture (Jakubus et al., 2019). In the following step, the solid particles that have taken up the analytes are separated through centrifugation and subsequently washed with an appropriate solvent to release the analytes. Consequently, in this technique, the choice of a suitable adsorbent is critically important. Metal-organic frameworks are an innovative class of nano adsorbents that are gaining attention due to their significant adsorption capacity, exceptional porosity, large surface area, impressive mechanical and thermal stability, and straightforward synthesis methods (Bazargan et al., 2021).

In this study, a combination of DSPE and DLLME based on the employment of magnetic ion fluid to extract Aflatoxin M1 (AFM1) and Ochratoxin A in pasteurized milk samples and then analyzing them with high performance liquid chromatography (HPLC) equipped with Fluorescence Detector was used. Furthermore, in this research the effect of the use of ultra violet waves in reduction of analyte content under study in the pasteurized milk samples are scrutinized for the first time.

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2-Material and method

- Sampling and chemical materials:

31 pasteurized cow milk samples were obtained in winter 2021 from local producers in East Azarbaijan province. These samples were kept in poly ethylene trephtalyte containers in refrigerator (4 degrees celcius) until the analysis time. One of the samples which did not have the studied analytes was chosen as blank and was used in improvement of the proposed method. All of the experiments were performed in Chemistry faculty of Tabriz University.

- Chemical materials:

Standard Aflatoxin M1, Ochratoxin A and three kinds of magnetic ion fluid including 1- hexyl- 3 methyl imidazolium tetra Choloro ferrate ([MIM][Fe Cl4C]), 1- butyl-3- methyl imidazolium tetra Choloro Ferrate ([MIM][Fe Cl4C]) and 1- butyl- 3 – methyl imidazolium di Bromo di Choloro Ferrate ([Br2MIM][FeCl4C]) were obtained from Sigma Company (Sigma, USA),O2H6,FeCl, 1,4- Banzen di carboxylic acid, Di Methyl Formamid, Aceton, Methanol, Isopropanol, Tri choloro acetic acid, Sodium Choloride, Sodium hydroxide from Merck Company (Merck, Germany) Acetonitril and water with LPLC- grade from Chem Lab Company (Chem Lab, Belgium).

- Analysis conditions with chromatography apparatus

Improving the analysis conditions and involved parameters in the process of separation using HPLC apparatus leads to the increase in the capacity of separation of chromatography system. Optimum conditions of HPLC equipped with FLD for analysis of aflatoxin M1 and Ochratoxin A is shown in Table 1.

Table 1- Optimum condition of HPLC-FLD

Type of column	Gemini C, Length 150 mm
Mobile phase	Acetonitrile: water (35:65 v/v)
Injector	Temprature: 40°c
Excitation wave length	360 nm for AFM1 and 333 nm for Ochratoxin A
Emission wave length	430 nm for AFM1 and 470 nm for Ochratoxin A
ID	4.6 mm
Acuracy flow	1.1 ml/min
Loop	20 µL
Particle size	5µm

- MOF synthesis

To synthesize metal – organic framework MIL - 88B (Fe) (Pezhhanfar et al., 2022), a mixture of 55 mL di methyl formemide and 4.4 mL of Sodium hydroxide solution (2 molar) is added to a glass container and the mentioned mixture is stirred until a homogenous solution is obtained, followed by adding 1.82 gr 1, 4- Banzen di carboxylic acid and 2.97 gr Fe Cl, O2H6 to the said solution and stirred for 15 minutes. Then the obtained solution is autoclaved at 100 degrees celcius for 12 hours. Then the acquired sediment is filtered using a filter paper and was dried in room temperature. Afterwards the solid powder was added to 200 mL of deionized water and stirred for one day. Finally the solution was

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filtered and MOF was dried for one hour in 15 mm Hg. It should be noted that the properties of synthesized MOF was discussed fully in our group's previous study (Pezhhanfar et al., 2022).

- **Optimizing the extraction conditions**

To reach high extraction efficiencies, the effect of different parameters like the amount of tri choloro acetic acid (from the amounts of 75 to 150 mg), duration of vortex (from the length of time between 1 to 5 minutes , amount of adsorbent (from the amount of 25 to 100 mg), the duration of adsorption (from the times between 1 to 5 minutes), the effect of adding salt (by adding between 0.0 to 10 percent w/v of sodium chloride salt), type (amongst four solvents of acetone, methanol, iso propanol and acetonitrile), the duration of desorption (from the duration of 1 to 5 minutes) and type (from the three magnetic ion fluid[M4IM][FeCl4C], [M4IM][FeCl6C], [Br2MIM][FeCl4C]), and volume (between volumes 55,60,65 and 70 µL) extracting solvents were investigated. In order to reach the maximum reduction of Aflatoxin M1 and Ochratoxin A content in samples, the effect of time of exposure to microwave radiation (15,30,45,60,75,90 and 120 seconds) and microwave power (from between the 0,90,180,270,360 and 450-watt electric power) was also investigated. In order to evaluate the effects of factors in the proposed method, the "One parameter in one time" method was used. The effect of these factors was evaluated by comparing the area under the peak produced from analytes in different conditions.

- **Disintegration characteristics**

In order to evaluate the presented method in this research, after sketching the calibration curve for each analyte, the linear range. Limit of detection, limit of quantitation, squared correlation coefficient, repeatability RSD %), efficiency and concentration factor was investigated and calculated.

- **Extraction method**

In this research a preparatory method based on the combination of DSPE and DLLME methods to extract Aflatoxin M1 and Ochratoxin A in pasteurized milk samples was developed. So, 7 mL of pasteurized milk sample was taken and vortexed for 3 minutes after adding 125 mg of tri chloro acetic acid. After centrifugation (with the speed of 5000 rpm for 5 minutes) the supernatant phase was discarded and 1 mL of acetonitrile was added to the absorbent particles. This mixture was vortexed for 4 minutes and again after centrifugation (with the speed of 5000 rpm for 5 minutes) the organic phase which has extracted anabollytes was taken and after mixing with 55 µL of magnetic ion fluid [M4IM] [FeCl6C] was injected to 5 mL of deionized water. Then in the presence of magnet the magnetic ion fluid containing the extracted analytes was collected in the tube and injected into HPLC-FLD apparatus in order to be quantitatively analyzed.

- **Investigating the effect of microwave irradiation on the content of mycotoxins**

In this step 7 mL of milk samples were taken and contaminated with the studied mycotoxins (200ng/L). Then the obtained specimen was subjected to microwave radiation, followed by extraction with previously said method.

3-Findings

-Optimization results on the effective factors in extraction

The amount of tri chloro acetic acid:

According to the obtained results in Diagram 1, with the increase in the amount of tri chloro acetic acid to 125 mg, the efficiency of extraction of the analytes under study was increased and then remains constant. As a result 125 mg was chosen as the best quantity of tri chloro acetic acid.

Duration of vortex:

According to the obtained results, with the increase in vortex time up to 3 minutes, the efficiency of extraction is increased and then remains constant.

Amount of absorbent:

The results obtained in Diagram 2 show that with the increase in the amount of absorbent (MOF)

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used up to 50 mg the efficiency of extraction of studied analytes is increased and then remains constant.

Duration of absorption:

Considering the obtained results, with the increase in the vortex time of the mixture of samples and absorbent particles up to 4 minutes,

without the increase in salting.

The type of solvent detergent:

The obtained results in Diagram 3 show that acetonitrile had the most efficacy in extraction of studied analytes in between the surveyed solvents and was chosen as optimum solvent for next stages.

Volume of acetonitrile:

Considering the obtained results (Diagram 4) with the increase of acetonitrile volumes up to 1 mL, extraction efficiency is increased and after that with the increase in volume, they decrease. As a result, 1mL of acetonitrile was chosen as optimum volume for the next studies.

The duration of desorption:

extraction of studied analytes is observed. Therefore, 55 µL was chosen as optimum volume.

The effect of microwave time in reduction of the concentration of analytes:

According to the results obtained in Figure 6 A, the concentration of analytes in the milk sample decreased for up to 90 seconds, after which no decrease in the concentration of analytes was observed.

efficiency of extraction is increased and then remains constant.

Effect of salting:

The obtained results show that increase of salting causes the reduction of efficiency of extraction of the studied analytes, and hence the next steps were taken

Considering the results, with the increase of the duration of vortexing the mixture of desorbing solvent and absorbing particles up to 4 minutes the efficiency of extraction is increased and then remains constant.

The type of extracting solvent:

As it can be seen in Diagram 5 magnetic ion fluid [M4IM] [FeCl6C] has higher extraction efficiency than the other solvents used and was chosen as optimum solvent for next stages.

Volume of extracting solvent:

Considering the results during DLLME process, with the increase in the volume of extracting solvent no significant change in efficiency of

The effect of microwave power in reduction of the concentration of analytes:

As can be seen in Diagram 6 B with the increase in microwave power to 360 watts (duration of exposure of samples to microwave for 90 seconds) concentration of analytes in the milk sample is reduced and after that, no decrease is observed in analyte concentration.

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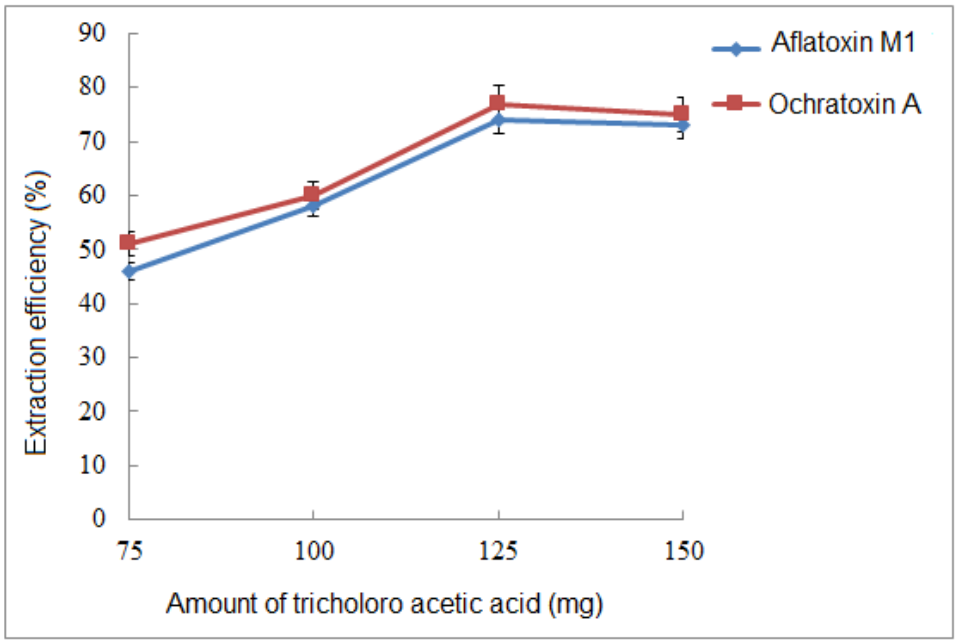

Diagram (1) - Optimization of the amount of tri chloro acetic acid

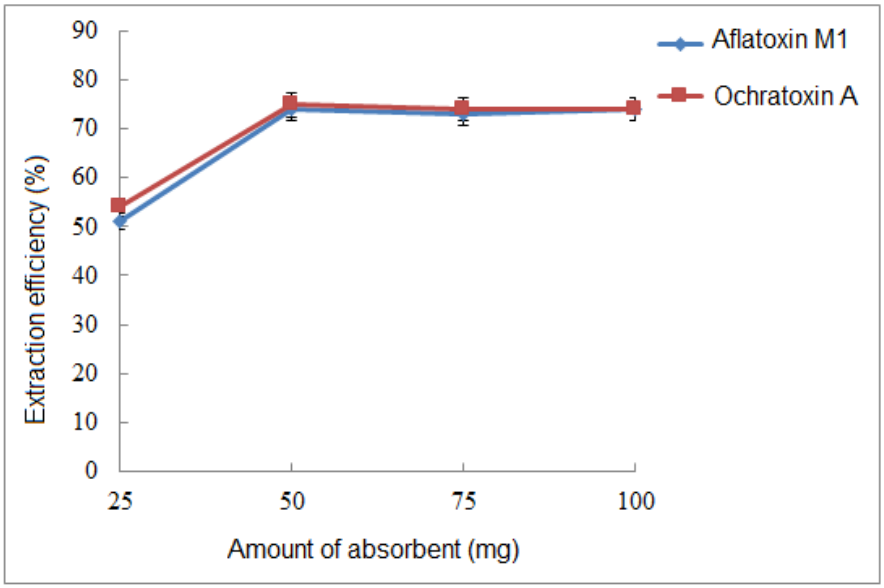

Diagram (2) - Optimization of the amount of absorbent

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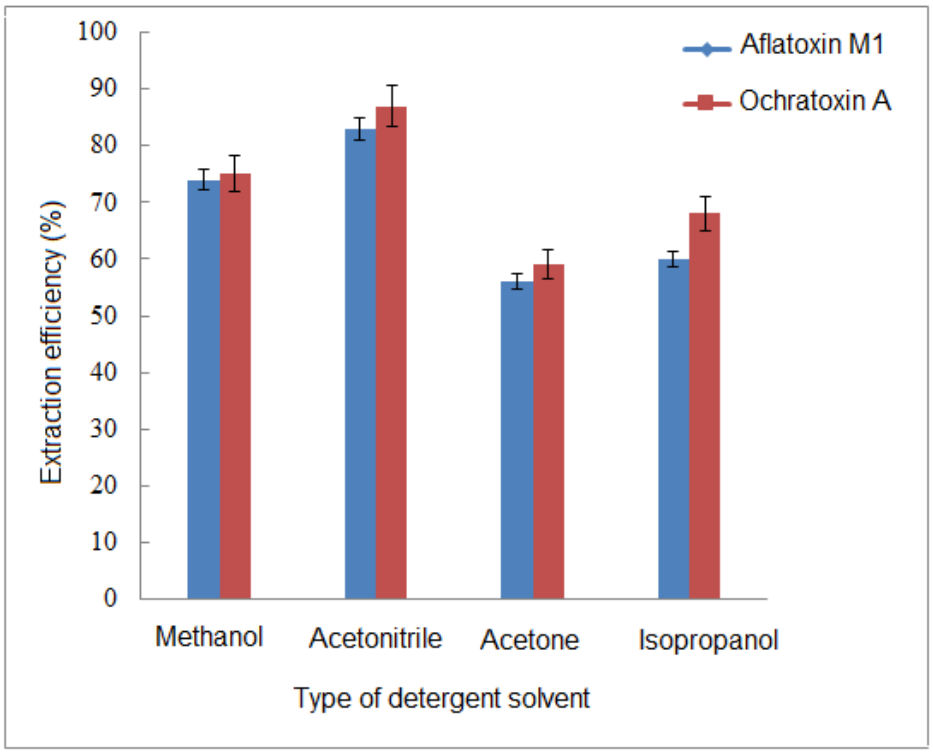

Diagram (3) - Choosing the type of detergent solvent

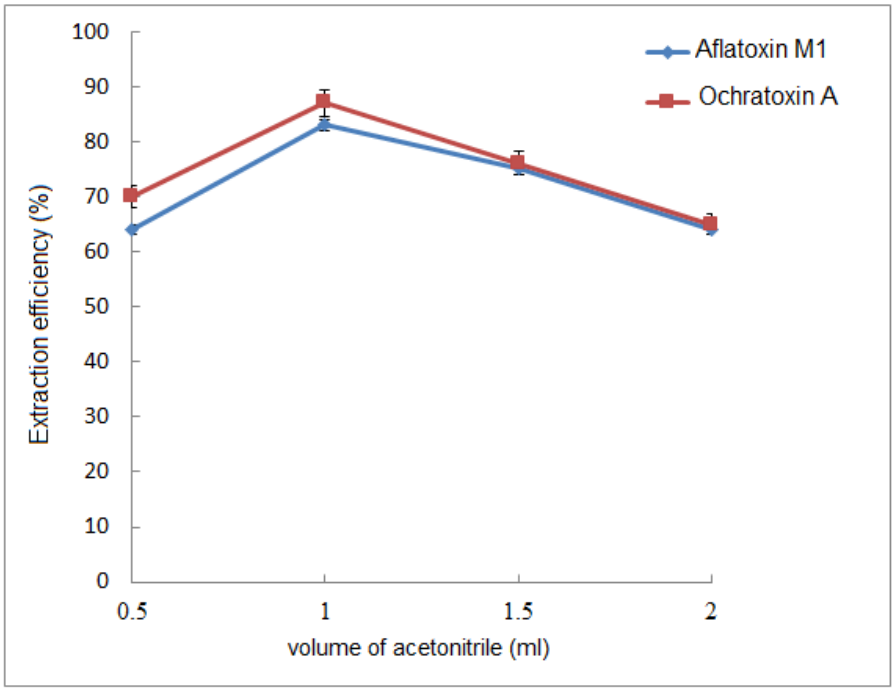

Diagram (4) - Optimization of the volume of acetonitrile

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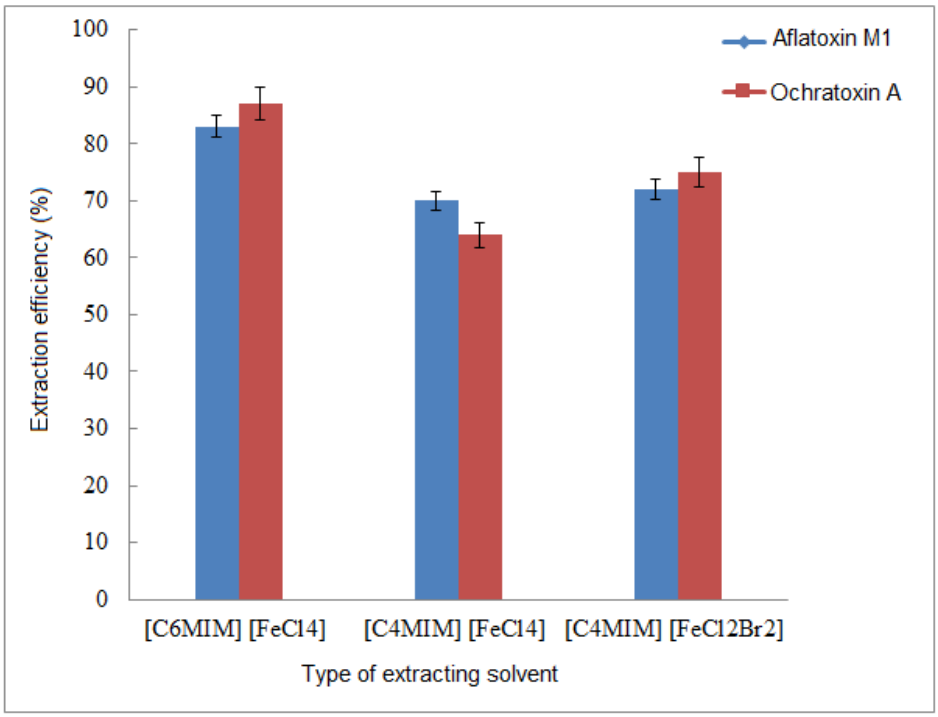

Diagram (5) - Choosing the type of extracting solvent

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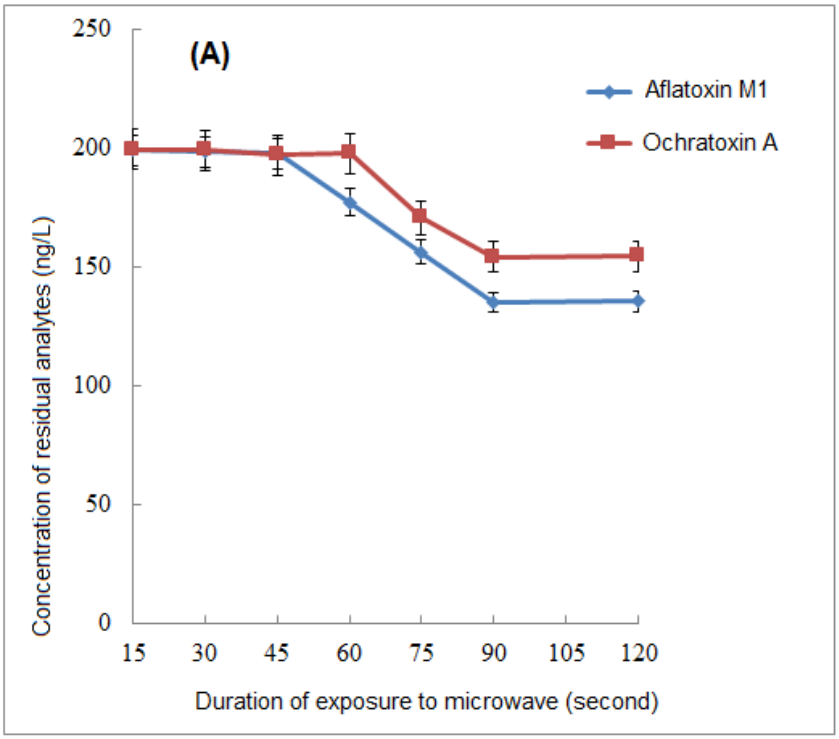

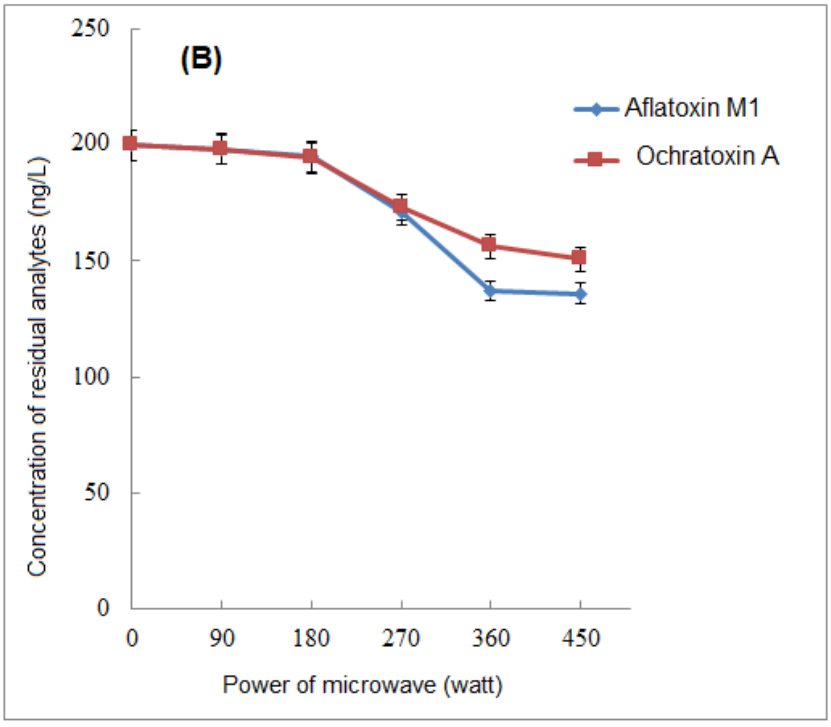

Diagram (6)- The effect of the duration of exposure to microwave radiation (A) and the power of microwave in reduction of the concentration of analytes (B)

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- **Disintegration characteristic results**

The competence figures of the presented method under optimum conditions were obtained. From sketching calibration diagram for each of the analytes the value of squared correlation coefficient (r^2) and linear range (LR) was obtained. In order to evaluate the repeatability and the precision of the method, relative standard deviation (RSD %) was used, Limit of detection (LOD) and Limit of Quantitation (LOQ) were considered against concentrations that the relation between signal and noise were 3 and 10 respectively. Calculation of concentration factor was done after comparing area under the peak of standard solution with peak area of analytes present in milk samples after performing the presented method and extraction, Competence figures of the presented method are given in Table 2.

Table (2) - Competence figures of the presented method

Analyte	Linear range (ng/L)	Detection limit (ng/L)	Quantitation limit (ng/L)	Squared correlation coefficient	Relative standard deviation (%) (n=6)	Extraction efficiency ± Standard deviation (n=6)	Condensation factor ± Standard deviation (n=3)
Aflatoxin M1	0.97-10^5	0.29	0.97	0.997	3.9	83 ± 3	232 ± 8
Ochratoxin A	0.77-10^5	0.23	0.77	0.998	4.2	89 ± 4	249 ± 11

- **Analysis results of real samples**

With analysis of real samples, application of the presented method in determining quantity of mycotoxins under study will be evaluated. So, the prepared milk samples were extracted under optimum conditions and were analyzed with HPLC-FLD. Cosidering the results obtained from 21 out of 30 milk samples, Aflatoxin M1 with the concentration of 23 to 196 ng/L was found. In 3 milk samples Ochratoxin A with a concentration range of 30 to 48 ng/L was found. Following this, the investigated samples were evaluated for the effect of microwave in reduction of analyte content were exposed to microwave irradiation (time and power of microwave were 90 seconds and 360 watts respectively). Noting the results obtained, microwave irradiation can reduce Aflatoxin M1 in the range of 27.6 to 33.5% and Ochratoxin A in the range of 17.3 to 20.8%.

- **Investigation of the effect of matrix in real samples**

In order to evaluate the effect of matrix in investigated milk samples, all samples and the blank sample were contaminated with Aflatoxin M1 and Ochratoxin A with concentrations of 10 and 15 ng/L. The values of relative recoveries obtained in Table 3 (in the range of 89 to 103%) shows that matrix of real samples has not much effect on the function of proposed method.

Table (3)- Investigation of the effect of matrix of pasteurized milk samples

Relative recovery ± standard deviation (=n3)

Analyte	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Spiked samples with every analyte with the concentration of 10 ng/L					
Aflatoxin M1	89±3	87±4	99±2	100±7	91±3

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Ochratoxin A	88±2	94±3	101±5	103±5	102±3
Spiked samples with every analite with the concentrations of 50 ng/L					
Aflatoxin M1	93±2	90±2	101±3	98±4	95±3
Ochratoxin A	93±3	96±4	103±3	100±2	103±4

4-Discussion and conclusion

In current study, a sample preparatory method based on the combination of DSPE and DLLME based on the use of optimized magnetic ion fluid was used to extract Aflatoxin M1 and Ochratoxin A in pasteurized milk samples. The extracted analytes were analyzed with HPLC – FLD. Furthermore, in this research the effect of the use of ultraviolet wave in reduction of the content of studied analytes in pasteurized milk samples were investigated.

Considering the complex matrix of milk sample, use of sedimentary agents and induction of protein sedimentation process in it before presenting disintegration method is an essential step. Considering the merits of Tri chloro acetic acid, it is used widely as sedimentary agent (Mohebbi et al., 2022). It must be noted that small amounts of Tri chloro acetic acid cannot cause all the proteins to settle and large amounts of it can denature and change the property of the sample so optimizing the amount of it is very important.

The length of time that sedimentary agent (Tri chloro acetic acid) is in contact with the milk sample is another important parameter that can influence the performance of the presented method (Mohebbi et al., 2022). In this study, vortex was used to increase the contact area between sedimentary agent and milk sample to hasten the sedimentation process of milk sample. Therefore the duration of vortex in this stage needs optimization.

The amount of absorbent in dispersive solid phase extraction is the most important parameter in the efficiency of the method. This effect in extraction efficiency is related to the ratio of solid phase of absorbent to the sample solution volume. In low quantities of solid the ratio of phase is small and extraction efficiency is low, but with the increase of absorbent the ratio of phase increases and extraction efficiency will be high (Jamali et al., 2013). Therefore the amount of absorbent must be optimized.

In the present method vortex is used to increase the contact area of analytes under study with absorbent and increase the analyte absorption rate by absorbent (Pezhhanfar et al., 2022). Therefore the duration of vortex is effective in efficiency of the proposed method and needs to be optimized.

Generally in extraction methods, increase of salt has two mutual effect on aqueous solutions. Firstly, the presence of salt causes the increase in ionic force of aqueous phase and hence, decrease the solubility of analytes in aqueous phase and increase their transfer to absorbent area and as a result increase extraction efficiency. Secondly, salt increase causes the increase in viscosity of aqueous phase and influences the dispersion coefficient of the analyte in aqueous phase that causes the reduction of extraction efficiency. Any of the effects that overwhelm the other the effect of that factor will defeat the other one (Mohebbi and Farajzadeh 2020). Therefore, one of the important parameters in most extracting methods that needs survey and optimization is the amount of salt.

In this method dispersive solvent in the dispersive liquid – liquid micro extraction phase as detergent solvent of analytes under study from absorbent is in dispersive solid phase extraction stage. Therefore, choosing the dispersive solvent is of great importance. The dispersive solvent in the offered method must have the ability of washing the absorbed analytes from absorbent and have the ability to mix with water (Wu et al., 2009). Hence, choosing the appropriate solvent is very essential.

The volume of used acetonitrile for washing analytes from absorbent is very important parameter in the proposed method. With the

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increase of the volume of detergent solvent/spreader analytes in the washing phase is diluted and in the DLLME phase, analytes are dissolved in the aqueous phase and the efficiency of the method is reduced. On the other hand, with the decrease of the volume of detergent solvent/ spreader analytes, the efficiency of the DLLME phase is reduced, because the extracting solvent are not dispersed as completely small droplets. Formation of cloud form causes the increase of contact area of extractor with aqueous phase and hasten the extraction of analytes into the extracting solvent (Wu et al., 2009). That's why, the volume of detergent solvent/spreader must be optimized.

After adding detergent solvent/ spreader onto the absorbent particles containing the absorbed analytes, the obtained mixture is vortexed for a while so that the process of desorption is hastened. Therefore, the duration of vortex at this stage is called desorption time and needs to be optimized.

In DLLME method, choosing the appropriate extracting solvent is the most important stage that can influence the efficiency of the method significantly (Daghi et al., 2022). In order to optimize this parameter, magnetic ion fluid, because of their low toxicity was chosen.

The volume of extracting solvent, is another parameter that can influence the extraction efficiency (Daghi et al., 2022). If the preliminary volume of the solvent is more, hence, the volume of the collected phase after extraction will be more also, and the concentration factor will be reduced and disintegrative signals will also be reduced. On the other hand, with the reduction of the volume of the solvent, efficiency of extraction and repeatability will be reduced, therefore, the volume of extracting solvent must be optimized.

The length of time of exposure to microwave irradiation and the power of microwave are also parameters that influence the remaining concentration of Aflatoxin M1 and Ochratoxin A in the milk samples. Increase in the length of time and power of microwave irradiation can influence the destruction and deformation of the form of compounds and cause the reduction of the remaining concentration of Aflatoxin M1 and Ochratoxin A.

Up to now different methods were employed to determine the amount of Aflatoxin M1 and Ochratoxin A in different samples. In order to assess the function and the success rate of proposed method, disintegration parameters related to it including LOD, LR, RSD, LOQ with the disintegration parameters if other methods used to determine Aflatoxin M1 and Ochratoxin A were compared. Considering the data in Table 4 proposed method of LOD and LOQ is lower than the other methods that reflects the sensitivity of the proposed method. Also, the suggested method has lower RSD and is repeatable. Linear range of the proposed method is also broader compared to the other methods. These results show that the proposed method has higher capability in measuring Aflatoxin M1 and Ochratoxin A.

Table (4)- Comparison of the efficiency of proposed method with other methods

Sample reference	Analyte relative SD (%)	Detection limit ng/l	Quantitation limit ng/l	Linear range method ng/l
Red wine (Marino-Repizo et al., 2015)	Ochratoxin A <9.16	130	410	- a
Mothers milk (Andrade et al., 2013)	AFM1 <9.5	10	30	100-15000 b
	Ochratoxin A <3.17	10	30	100-15000 c
Milk	AFM1 12.3	60	210	210-5000 c

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(Huang et al., 2015)				
Milk (present study)	AFM1: 3.9	0.29	0.97	0.97-10^5
	Ochratoxin A: 4.9	0.23	0.77	0.77-10^5

*Solid phase extraction coupled with liquid chromatography equipped with consecutive mass detector

*Liquid-liquid extraction coupled with liquid chromatography equipped with fluorescence detector

*Liquid phase extractor with hallow fiber coupled with liquid chromatography equipped with consecutive mass detector

In the present study, a combination of DSPE and DLLME methods as a new and efficient method of preparation to extract Aflatoxin M1 and Ochratoxin A in pasteurized milk samples was used. The proposed method was fast and ecofriendly and considering the obtained results has higher capability in extracting Aflatoxin M1 and Ochratoxin A in pasteurized milk samples (high extraction efficiency, low limit of detection and limit of quantitation and appropriate repeatability). Also, despite the presence of different organic and mineral compounds in milk texture, the sample matrix has no tangible effect on the efficiency of the proposed method.

5-Conflict of interests

Authors of this paper have no conflict of interests.

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اندازه گیری میزان آفلاتوکسین M_1 و اکراتوکسین A در نمونه های شیر پاستوریزه و بررسی تاثیر امواج مایکروویو در کاهش غلظت آن‌ها

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چکیده

با توجه به گستردگی استفاده از محصولات لبنی مانند شیر پاستوریزه، این محصولات باید عاری از هر گونه آلاینده مانند باقیمانده دارو، آفت کش، مایکوتوکسین و غیره باشند. مایکوتوکسین‌ها (مانند آفلاتوکسین‌ها، اکراتوکسین A و غیره) دسته‌ای از مواد سمّی و آلاینده مواد غذایی، خوراک دام و طیور هستند که به طور طبیعی از انواع خاصی از قارچ‌ها تولید می‌شوند. حضور مایکوتوکسین‌ها در شیر برای سلامتی مصرف کننده بسیار خطرناک است. بنابراین توسعه روش‌های سریع، کارآمد و آسان برای بررسی حضور مایکوتوکسین‌هایی مانند آفلاتوکسین M_1 و اکراتوکسین A در مواد غذایی بسیار حائز اهمیت می‌باشد. در این کار پژوهشی یک روش تجزیه‌ای کارآمد مبتنی بر استخراج فاز جامد پخشی ترکیب شده با میکرواستخراج مایع-مایع پخشی برای استخراج آفلاتوکسین M_1 و اکراتوکسین A از نمونه های شیر پاستوریزه و آنالیز آن‌ها با کروماتوگرافی مایع با کارایی بالا مجهز به دتکتور فلورسانس ارائه شده است. تحت شرایط بهینه حدود تشخیص و اندازه گیری به ترتیب در محدوده های ۰/۲۹-۰/۲۳ و ۰/۹۷-۰/۷۷ نانوگرم بر لیتر حاصل شدند. همچنین فاکتور تغلیظ و راندمان استخراج به ترتیب در محدوده های ۲۴۹-۲۳۲ و ٪ ۸۹-۸۳ بدست آمدند. در تعدادی از نمونه ها نیز آفلاتوکسین M_1 (در ۷۰ درصد از نمونه های شیر بررسی شده) و اکراتوکسین A (در ۱۰ درصد از نمونه های شیر بررسی شده) یافت شد. همچنین با توجه به نتایج بدست آمده، تابش مایکروویو می‌تواند میزان آفلاتوکسین M_1 (در محدوده ۲۷/۶ تا ۳۳/۵ درصد) و اکراتوکسین A (در محدوده ۱۷/۳ تا ۲۰/۸ درصد) کاهش دهد.

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