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Determination of Aflatoxin M_1 in milk by High Performance Liquid Chromatography in Mashad-Iran in 2014

Mohamadi Sani A*, Mohseni Sh., Mohamadi R.

Department of Food Science and technology, Quchan Branch, Islamic Azad University, Quchan, Iran

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ABSTRACT

The aim of this study was to evaluate Aflatoxin M1 (AFM_1) contamination in milk samples in Mashad-Iran during January-April 2014. Forty six milk samples were collected from retail stores. The occurrence and concentration range of AFM_1 in the samples were investigated by High Performance Liquid Chromatography (HPLC) method. AFM_1 was found in 100% of the examined milk samples by average concentration of 55.7 ng/kg and contamination level ranging between 12 and 110ng/kg. The concentration of AFM_1 in all of the samples were lower than Iranian national standard and FDA limit (500ng/L) and in 28 (51%) of the samples AFM_1 concentration was greater than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission.

KEYWORDS: Aflatoxin M1, Milk, HPLC, Food safety, Mashad.

INTRODUCTION

Mycotoxins are secondary metabolites of molds which are associated with certain disorders in animals and humans. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer and it is this aspect which has evoked global concern over feed and food safety, especially for milk and milk products [1]. Aflatoxin M1 (AFM_1) is a hepatocarcinogen found in milk of animals that have consumed feeds contaminated with aflatoxin B1 (AFB1), the main metabolite produced by fungi of the genus *Aspergillus*, particularly *A. flavus*, *A. parasiticus* and *A. nomius* [2]. About 0.3–6.2% of AFB1 in animal feed is transformed to AFM_1 in milk [3]. Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country [4]. The European Community prescribes that the maximum level of AFM_1 in liquid milk should not exceed 0.05µg/kg. However, according to the US standard, the level of AFM_1 in liquid milk should not be higher than 0.5µg/kg [5].

The objective of this study was to evaluate the occurrence of AFM_1 using High Performance Liquid Chromatography (HPLC) method in milk distributed in Mashad-Iran during **January-April 2014**.

MATERIALS AND METHODS

Materials

Samples

In this study the levels of AFM_1 in pasteurized milk samples intended to distribute in retail stores of Mashad-Iran was determined in **January-April 2014**. Forty six milk samples (1000 ml milk packet) were collected by simple random sampling method. The samples were transported to the laboratory in an insulated container at about 4 °C and analyzed upon arrival.

Chemicals and standards

AFM_1 standard was obtained from Sigma Chemical (St Louis, MO, USA). AFM_1 stock solution was prepared in acetonitrile, at a concentration of 10 µg/ml. HPLC grade solvents were purchased from Fisher Scientific (New Jersey, USA). The immunoaffinity columns AflaM1™ HPLC were obtained from VICAM (Watertown, MA, USA). The water was double distilled with Millipore water purification system (Bedford, MA, USA) and was used for analysis. Trifluoracetic acid (TFA) was supplied from Sigma.

Methods

Determination of AFM_1

The method used for determination of AFM_1 was the AOAC Official Method 2000.08 reported by Dragacci, Grosso, and Gilbert (2001) [6].

* **Corresponding Author:** Mohamadi Sani A., Department of Food Science and technology, Quchan Branch, Islamic Azad University, Quchan, Iran

Extraction procedure

Briefly, 100 ml of milk samples warmed at 37Ć and centrifuged at 4000 rpm for 5 min. Then the fat layer was removed completely and the skim milk was filtered through Whatman No. 4 filter paper. The filtrate was gathered in flask and loaded into immunoaffinity chromatography column. Then 50 ml of the filtrate was taken in a syringe barrel which was attached with immunoaffinity columns (IAC). The test portion was passed at the flow rate of 2–3 mL/min. Tow times of 10 ml purified water was passed through the column at a rate of 2–3 mL/min. AFM_1 was eluted with 1.5 ml of acetonitrile-methanol (3+2, v/v) and collected in a glass tube. The gentle stream of nitrogen was passed to evaporate the eluate to dryness. Recovery tests using IACs were performed to determine the efficacy of the analytical method by spiking raw milk with AFM_1 standard solution at the levels of (0.01, 0.02, 0.03, 0.05, 0.1, 0.5 µg/L) and submitting them to free extraction procedures. AFM1 standard was purchased from Sigma Chemical Co (St Louis, MO, USA).

LC Determination with fluorescence detection

The HPLC system of Agilent 1200 series (Waldbonn, Germany), equipped with a thermostated auto-sampler and a fluorescence detector FLD G1321A with excitation and emission wavelength of 365 nm and 435 nm, respectively, and a Inertsil ODS-3 (250 × 4.6 mm, 5 µm) operating at a flow-rate of 1.0 $mLmin^{-1}$ in isocratic elution with a mixture of water: methanol: acetonitrile (70:15:15, v/v/v) was used for AFM_1 determination. The system was interfaced, via network chromatographic software (Agilent ChemStation), to a personal computer for instrumentation control, data acquisition and processing. Standard solutions AFM_1 with concentrations of 0.05, 0.5, 0.7 and 1.0 ng/mL in acetonitrile were used to obtain the calibration curve. The retention time for AFM_1 was 20 min.

RESULTS AND DISCUSSION

The standard solutions of concentration from 0.05ng/mL to 1ng/mL AFM1 were used to find calibration/standard curve as described by the following regression equation: $y= 29.77x-3.75e^{-1}$ where y = area and x = amount of AFM1. The results showed the linearity of the standard curve over the range studied. The coefficient of determination (R2) was 0.9993. Figure 1 gives the calibration curve of standard solutions of AFM1 with concentrations of 0.05, 0.5, 0.7 and 1.0 ng/mL by HPLC analysis.

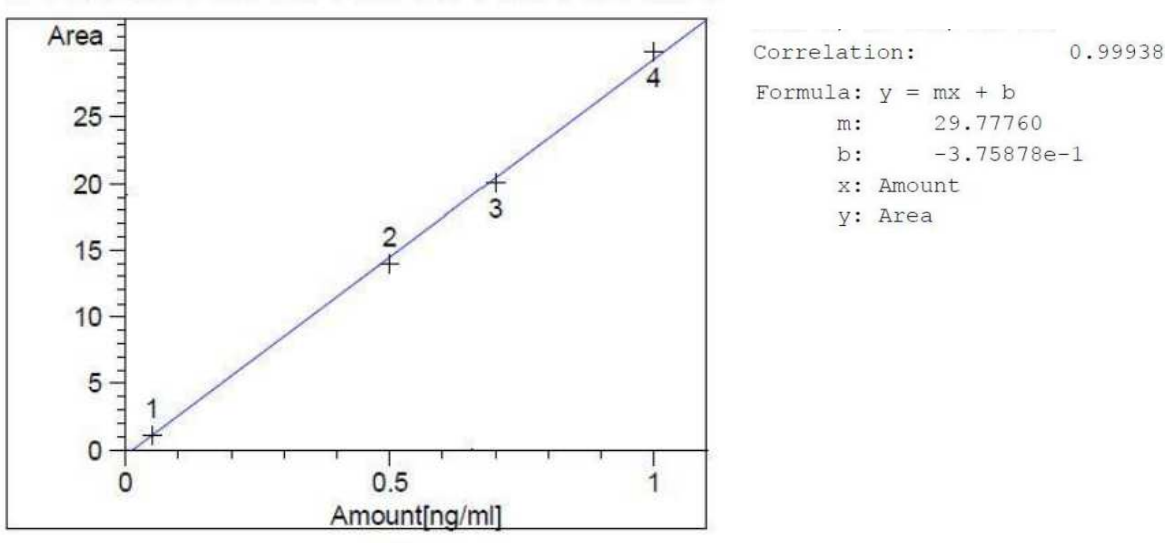

Figure1. Calibration curve of standard solutions of AFM_1 with concentrations of 0.05, 0.5, 0.7 and 1.0ng/ml by HPLC analysis.

Analytical results showed that the incidence of AFM_1 contamination in pasteurized milk samples was low. Although all the samples were contaminated with AFM_1, the toxin concentration was lower than Iranian national standard and FDA limit (500ng/L) and in 28 (51%) of the samples AFM_1 concentration was greater than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission. The Table1 shows the range of contamination level in different milk samples. The minimum and maximum contamination level

of AFM_1 was found to be 12 and 110ng/L milk respectively. The mean AFM_1 level in the analyzed samples of pasteurized milk was 55.7ng/L.

Table1. Distribution of milk samples and AFM_1 concentration (ng/L)

Samples	AFM_1 **Content**	Samples	AFM_1 **Content**
1	88	24	78
2	96	25	45
3	23	26	12
4	16	27	32
5	12	28	65
6	110	29	85
7	14	30	95
8	19	31	65
9	55	32	45
10	45	33	72
11	47	34	26
12	89	35	38
13	85	36	85
14	36	37	45
15	65	38	96
16	45	39	81
17	19	40	14
18	98	41	54
19	56	42	53
20	32	43	67
21	65	44	84
22	54	45	16
23	78	46	65
X	55.7	SD	27.9

The mean AFM_1 concentrations in milk in European, Latin American and Far Eastern diets have been reported by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001) to be 23, 22 and 360ng/L, respectively [7]. Thus, the observed mean AFM_1 concentration in Mashad milk samples was as high as the European and Latin American and much lower than those reported for the Far Eastern diets.

Table2. The incidence of milk contamination in Iran in other studies

Location	Reference	Method of detection	Sample size	Percent of contamination	Percent of contamination >50ng/L	AFM_1 concentration (ng/L)
Mashad (North east of Iran)	Mohamadisani etal., 2010	ELISA	196	100	80.6	77.92
Five states of Iran	Tajkarimi et al., 2007	HPLC	98	100	37.7	39
Tehran (Capital of Iran)	Heshmati & Milani 2009	ELISA	210	55.2	33.3	58
14 states of Iran	Tajkarimi et al., 2008	HPLC	319	54	23	57
Shiraz (South of Iran)	Alborzi et al., 2006	ELISA	624	100	17.8	n.r*
Ahwaz (South of Iran)	Rahimi et al., 2010	ELISA	311	42.1	12.5	43.3
Sarab (North west of Iran)	Kamkar 2005	TLC	111	76.6	40	61.43
Central part of Iran	Fallah 2010	ELISA	225	67.1	33.1	49.9
Ardabil (North west of Iran)	Nemati et al., 2010	ELISA	90	100	33	n.r*

***not reported.**

In another hand, several studies have been done to determine AFM_1 contamination of milk in Iran (Table 2). The incidence of AFM_1 observed in the present study was lower than the incidence of AFM_1 reported by other authors [8, 9, 10, 11, 12, 13, 14, 15, 16]. The variations may be attributed to differences in region, season and specially analysis method. Based on the above results, the present situation is hopeful and might represent the possibility of altering standard limit of AFM_1 concentration in milk in Iran.

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