School and Health 21, 3/2008, *Social and Health Aspects of Health Education*

THE FIRST RESULTS OF ANALYSES OF OCHRATOXIN A IN HUMAN MILK IN SLOVAKIA

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Abstract: *The authors analysed samples of human milk with a view to find potential contamination with a mycotoxin - ochratoxin A. A rapid and effective separative method the HPLC was used. Donors offered their human milk voluntarily and it was taken right at the clinic of children. The results of such kind of analyses in our country are summarized in the table. The analysis conditions are added to the data obtained by the referred chromatographic method.*

Keywords: *human milk, ochratoxin A, HPLC analysis, results of analyses*

Introduction

From lots of xenobiotics occuring in food, ochratoxin A is one of serious mycotoxins. The mycotoxin is produced by moulds growing and proliferating particularly on several kinds of food. Cereals, that are base material to production of food everyday eaten, are hospitable media.

Mycotoxins can be excreted from the body also to human milk. Human milk can be contaminated in dependence on environment and food pollution with xenobiotics.

Conditions of qualitative and quantitative determination of ochratoxin A

To carry out the determination of ochratoxin A by the HPLC technique, the chromatografic system Merck Hitachi with fluorescence detector L-7480 was used.

Procedure

A test portion of the human milk sample was extracted by n-hexan to remove the lipid fraction. In order to take out ochratoxin A, extraction with 1 % $NaHCO_3$ as the elution solution followed. A filtrated portion mixed with phosphate buferred saline was passed through the immunoaffinity column (IAC) specific to the mycotoxin where the analyte was adsorbed, purified and consequently eluated.

Ochratoxin A was separated from the cleaned-up and concentrated-up extract by means of the HPLC system with reverse phase separating column and detection of the analyt was carried out through the use of fluorescence detector.

HPLC operating conditions

Column oven temperature:	35 °C
Analytical separating column:	LiChroCART 125-4 filled with LiChrospher RP-18
Mobile phase:	acetonitrile – water - acetic acid with parts per volume 51 : 47 : 2
Flow rate mobile phase:	1,0 ml/min
Fluorescence detection	excitation wavelenth – 333 nm emission wavelenth – 433 nm
Injection volume:	80 µl

Results of analyses of ochratoxin A in human milk

Tables of ochratoxin A values found in human milk samples and comment notes to abbreviation

Values of ochratoxin A in human milk samples				
N°	Volume of test portion of sample taken for analysis [ml]	Number of IAC	Concentration of ochratoxin A [ng/l]	Date of analysis
1	60	2	9,7	05.04.07
2	25	1	ND (4,8)	29.03.07
3	50	2	ND (4,8)	05.04.07
4	30	1	ND (4,8)	29.03.07
5	70	3	ND (4,8)	05.04.07
6	25	1	ND (4,8)	14.08.07
7	60	2	ND (4,8)	11.04.07
8	60	2	2,3	11.04.07
9	50	2	47,6	23.03.07
10	25	1	ND (4,8)	29.03.07
11	25	1	ND (4,8)	14.08.07
12	25	1	ND (4,8)	14.08.07
13	25	1	ND (4,8)	14.08.07
14	25	1	ND (4,8)	16.08.07
15	25	1	ND (4,8)	14.08.07
16	25	1	ND (4,8)	14.08.07
17	25	1	< 14,4 (6,4)	14.08.07

18	25	1	ND (4,8)	14.08.07
19	25	1	< 14,4 (5,7)	14.08.07
20	25	1	ND (4,8)	16.08.07
21	25	1	ND (4,8)	16.08.07
22	25	1	ND (4,8)	16.08.07
23	25	1	< 14,4 (5,3)	16.08.07
24	25	1	ND (4,8)	16.08.07
25	25	1	ND (4,8)	14.08.07
26	25	1	ND (4,8)	14.08.07
27	50	1	5,0	14.08.07
28	50	1	5,4	14.08.07
29	25	1	< 14,4 (6,8)	14.08.07
30	50	1	0,6	14.08.07
31	25	1	ND (4,8)	14.08.07
32	25	1	ND (4,8)	14.08.07

Quality assurance					
Test material		Ochratoxin A			
		Assigned value µg/kg	Satisfactory range µg/kg	Measured value µg/kg	
1	T1742 Baby Food (FAPAS®)	0,60	0,34 - 0,87	0,49	81,7
				0,54	90,0
2	Spiking of human milk matrix with ochratoxin A solution				

Comment notes		
ND (4,8)	Value equal or less than LOD	
< 14,4	Value less than LOQ but higher than LOD	
LOD	Limit of detection of the analytical method	LOD = 4,8 ng/l *
LOQ	Limit of quantification of the analytical method	LOQ = 14,4 ng/l *
	* If the volume of test portion of sample taken for analysis is 25 ml	

Conclusions

Human milk can be contaminated with various substances dangerous to health and it is not possible to present the whole scale of the them. With respect to the fact that

human milk as the first nutrition of a child influences significantly its physiogeny, studies from our country as well as from foreign countries are focused on presence of not only the most toxic xenobiotics but less toxic ones as well.

Within the scope of our research we concerned in the nephrotoxic mycotoxin ochratoxin A. There are not only our first results but also the first findings in Slovakia. Up to now our determinations enable to claim they are not alarming. We have found the maximum value of ochratoxin A 47,6 ng per litre of human milk whereas presence of the toxin was proved in 31% of the total number 32 analysed samples. Our experiments with determination of ochratoxin A continues.

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The work is a part of the project VEGA N^{o} 1/4247/07 „Nutrition of mothers in relation to presence of selected mycotoxins in human milk and their influence on clinical parameters of newborns and breastfed infants“.

PRVÉ VÝSLEDKY ANALÝZY OCHRATOXÍNU-A V MATERSKOM MLIEKU NA SLOVENSKU

Súhrn: Autori analyzovali vzorky materského mlieka na zistenie možnej kontaminácie mykotoxínom ochratoxín A. Použili rýchlu a vysoko účinnú separačnú metódu kvapalinovej chromatografie HPLC. Materské mlieko poskytli matky dobrovoľne a bolo odobraté priamo na detskej klinike. Výsledky prvých analýz tohto druhu u nás sú zhrnuté v tabuľke. K údajom získaným uvedenou chromatografickou metódou sú doložené podmienky analýz.

Kľúčové slová: materské mlieko, ochratoxín A, analýza HPLC, výsledky analýz