World Academy of Science, Engineering and Technology
International Journal of Pharmacological and Pharmaceutical Sciences
Vol:6, No:12, 2012

Analysis of Roasted and Ground Grains on the Seoul (Korea) Market for Their Contaminants of Aflatoxins, Ochratoxin A and *Fusarium* Toxins by LC-MS/MS

So-young Jung, Bu-chuhl Choe, Gi-young Shin, Jung-hun Kim, and Young-zoo Chae

***Abstract*—**A sensitive and specific method for quantitative determination of aflatoxins(B1, B2, G1,G2), deoxynivalenol, fumonisin(B1,B2), ochratoxin A, zearalenone, T-2 and HT-2 in roasted and ground grains using liquid chromatography combined with tandem mass spectrometry. A double extraction using a phosphate buffer solution followed by methanol was applied to achieve effective co extraction of 11 mycotoxins. A multitoxin immunoaffinity column for all these mycotoxins was used to clean up the extract. The LODs of mycotoxins were 0.1~6.1 µg/kg, LOQs were 0.3~18.4 µg/kg. Forty seven samples collected from Seoul (Korea) for mycotoxin contamination monitoring. The results showed that the occurrence of zearalenone and deoxynivalenol were frequent. Zearalenone was detected in all samples and deoxynivalenol was detected in 80.9 % samples in the range 0.626 ~ 29.264 µg/kg and N.D ~ 48.332 µg/kg respectively. Fumonisins and ochratoxin A were detected in 46.8% samples and 17 % samples respectively, aflatoxins and T-2/HT-2 toxins were not detected all samples.

***Keywords*—**LC-MS/MS, mycotoxins, roasted and ground grains.

I. Introduction

MYCOTOXINS, a series of secondary metabolites generated from molds, widely contaminate plant origin product such as crop, food and feeds. The contamination of foods and feeds by the major mycotoxins, e.g. aflatoxins(AFs), ochratoxin A(OTA), fumonisins(FBs), deoxynivalenol (DON), T-2 toxin(T-2),HT-2 toxin(HT-2), and zearalenone (ZEA), has been recently recognized by the World Health Organization as a significant source of food borne illnesses[1].

Since the recognition of mycotoxins as a public health problem in the late 1950s, the measurement of these contaminants has been the subject of constant improvement in analytical technology. The techniques have established from thin-layer chromatography (TLC), which is still a viable procedure, to the currently favored techniques of liquid chromatography (LC) and enzyme-linked immunosorbent assays (ELISA). However, the occurrence of pseudo-positive results and sometimes unacceptable quantification accuracy restrict its futher application. The confirmatory quantification of mycotoxins includes thin layer chromatography(TLC) [2], [3], gas chromatography (GC) [4], [5] and high performance liquid chromatography (HPLC) in combination with various detectors (diodearray[6], [7], flurorescence[8], [9] and MS/MS[10], [11]). Most of recently developed methods use commercial immunoaffinity column for the clean up following solvent extraction. Commercial multimycotoxin immuaffinity columns have been successfully used for the simultaneous determination by liquid chromatography with MS/MS [12]. The quantitative method has many advantages including simple preparation, rapid determination and high sensitivity, which could be applied to the determination of multi-component mycotoxin contaminants in complex matrixes.

Roasted and ground grains are called ‘Misugaru’ in Korea, used in a cold beverage or instead of a simple meal. The aim of this study is establish a reliable and rapid LC-MS/MS method for the simultaneous analysis of 11 kinds of mycotoxins in roasted and ground grains mainly considering the actual contaminant situations in Seoul (Korea).

So-young Jung is with the Seoul Metropolitan Government Research Institute of Public Health and Environment, Seoul, Korea , (phone: 82-2-570-3247; fax: 82- 2-570-3248; e-mail: syjung12r@ seoul.go.kr).

II. Procedure

A. Chemicals and Materials

Methanol (HPLC grade) were purchased from from Fisher Scientific Korea Ltd., Ammonium acetate, ZEA(100 µg /mL), DON(100 µg /mL), OTA(10 µg /mL), FBs(1 mg) were from Sigma-Aldrich. T-2/HT-2(100 µ g/mL) toxin were purchased from Biopure . Aflatoxin Mix(cat.No.46304-U) were obtained from Spelco. Multimycotoxin immuaffinity column was MYCO 6 in1 (Vicam Co., USA). Phosphate buffered solution(PBS) at pH 7.4 solution was prepared by dissolving 0.2 g KCl, 8.0g NaCl, 0.2 g KH_2PO_4, 2.92 g $Na_2HPO_4 \cdot 12H_2O$ in 1 L distilled water.

B. Equipment and Instrumental Analysis

LC-MS/MS analyses were performed on a Qtrap-3200 Triple-Quadrupole tandem mass sapectrometer(Applied Biometrics, Bonn, Germany) with Agilent 1200 series liquid chromatograph (Agilent, Palo Alto, CA, USA). The analyte were separated by Luna C18 column and eluted with 5mM ammonium acetate, 1% acetic acid/distilled water and methanol for the ESI^+ and ESI^- analysis. The analysis condition was described Table I.

TABLE I
THE OPERATION PARAMETERS OF LC-MS/MS FOR ANALYSIS OF MYCOTOXINS

Instrument	Parameter	Conditions
LC	Column	Luna C18(2) (150 x 3.0 mm x 3 μ m)
	Mobile phase	A : Water (5mM Ammonium Acetate + 1 % Acetic acid)
		B : Methanol (5mM Ammonium Acetate + 1 % Acetic acid)
	Gradient	Time (min) 0 3 5 10 18 23 25 30
		Solvent A (%) 70 70 60 30 30 20 70 70
	Flow rate	0.3 mL/min
	Injection volume	20 μ L
MS	Curtain gas	30.0
	Ionspray voltage	-4500(Negative), 5500(Positive)
	Temperature	500
	Ion Source Gas 1	50
	Ion Source Gas 2	50

C . Sample Preparation

Ground samples (3 g) were extracted with 30 mL PBS solution, by 30min on shaker. After centrifugation at 12000 rpm for 15min, 21 mL of PBS extract were filtered (*extract A*). Then 21 mL methanol were added to the residues, and sample was extracted again by shaking for 30 min (in this way the extraction solvent was about 70% methanol). After centrifugation, 3 mL of 70% methanol/PBS extract were diluted with 27 mL PBS solution and filtered (*extract B*). 30 mL *extract B* were pass through the MYCO 6 in 1 column at 1~2 drop per second and washed with 20 mL PBS to remove methanol residues. After passing through 3 mL *extract A*, the column was washed with 10 mL distilled water. Toxins were eluted with 3 mL methanol in two steps of 1.5 mL each 1 drop per second. After first step, 5 min interval was allowed. The eluate was evaporated under an air stream at 50 ℃ and reconstituted with 500 μL mobile phase.

III. RESULT AND DISCUSSION

The stock solution of 11kinds of mycotoxin standards were prepared with mobile phase (A:B=50:50). As for the selection of parent ions, the ionization mode (ESI+/ESI-) should first be decided according to characteristics of mycotoxins. Based on the confirmation of parent ions, two daughter ions should be selected. Therefore, the optimization of daughter ions and their collision energy was performed under daughter scan. The final daughter ions and optimal collision energy was shown in Table II.

Fig. 1 is the chromatograms of the 11 mycotoxin standards uing condition of Table II.

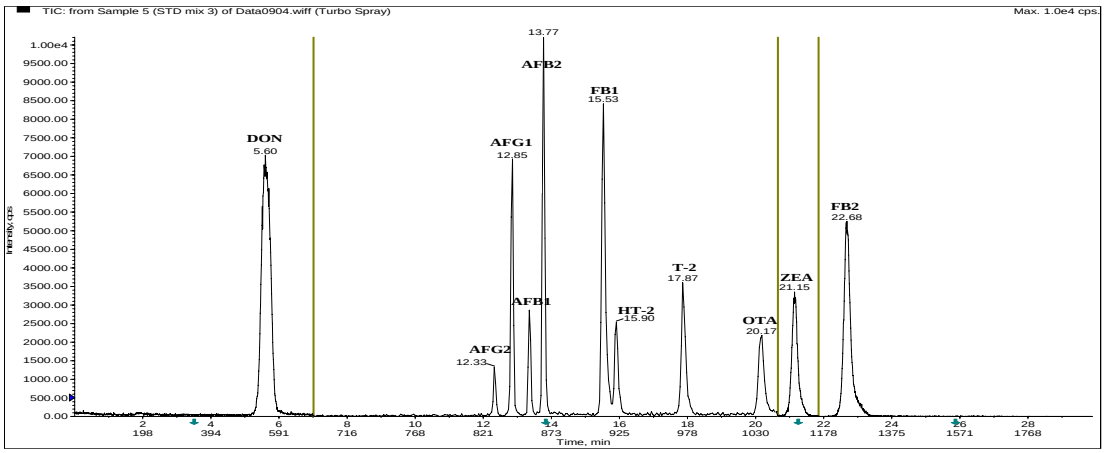

Fig. 1 LC-MS/MS chomatogram of 11 mycotoxins standard
(DON 100 μg/kg, AFG1,AFG2 3 μg/kg, AFB1,AFB2, HT-2, T-2, OTA, ZEA 10 μg/kg, FBs 100 μg/kg)

TABLE II
MS/MS PARAMETER FOR MYCOTOXIN DETECTION BY THE MULTIPLE REACTIONS MONITORING (MRM) METHOD

Analyte	Formula	Precursor ion	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
DON	$C_{15}H_{20}O_6$	$[DON+CH3COO]^-$	355	59 295	-20	-3.0	-34 -12	0 -4.0
AFG_2	$C_{17}H_{14}O_7$	$[AFG_2+H]^+$	331	313 245	66	6.0	27 39	4.0 4.0
AFG_1	$C_{17}H_{12}O_7$	$[AFG_1+H]^+$	329	243 200	76	4.0	37 51	4.0 4.0
AFB_2	$C_{17}H_{14}O_6$	$[AFB_2+H]^+$	315	287 259	66	2.5	31 39	4.0 4.0
AFB_1	$C_{17}H_{12}O_6$	$[AFB_1+H]^+$	313	241 128	61	7.0	49 83	4.0 4.0
HT-2	$C_{22}H_{32}O_8$	$[HT-2+NH_4]^+$	442	263 105	21	5.0	19 57	4.0 4.0
T-2	$C_{24}H_{34}O_9$	$[T-2+NH_4]^+$	484	215 185	26	5.5	25 27	4.0 4.0
FB_1	$C_{34}H_{59}NO_{15}$	$[FB_1+H]^+$	722	334 352	71	8.0	49 45	4.0 4.0
FB_2	$C_{34}H_{59}NO_{14}$	$[FB_2+H]^+$	706	336 318	76	8.0	45 55	4.0 4.0
OTA	$C_{20}H_{18}ClNO_6$	$[OTA+H]^+$	404	239 102	41	6.5	31 93	4.0 4.0
ZEA	$C_{18}H_{22}O_5$	$[ZEA-H]^-$	317	175 130	-55	-4.0	-31 -25	0 0

A calibration curve was plotted using standards at concentrations in Table III. The linear equation of the calibration curve and R2 value, as well as the limit of detection and quantitation of the analysis method of mycotoxins established in this study is shown in the Table III. LODs and LOQs were determined with the ICH Q2B guideline [13]. The calculation is based on the standard deviation of the response (σ) and the slope of the calibration curve(S) at levels approaching the limits according to equation 1 and 2:

$$LOD = 3.3 (\sigma / S) \quad (1)$$

$$LOQ = 10 (\sigma / S) \quad (2)$$

The standard deviation of the response can be determined based on the standard deviation of the y intercepts of regression line.

TABLE III
LINEAR RELATION AND SENSITIVITY OF MYCOTOXINS

Analyte	Lineaty range (μ g/kg)	Calibration curve	Coefficient	LOD (μ g/kg)	LOQ μ g/kg)
DON	10~1000	y=929x + -167	1.0000	6.1	18.4
AF G2	0.3~30	y=0.0011x -120	0.9998	1.3	3.8
AF G1	0.3~30	y=0.0061x +0.0024	0.9994	0.3	1.0
AF B2	1~100	y=673x +816	0.0097	1.3	4.0
AF B1	1~100	y=0.0026x +0.0032	0.9996	0.1	0.3
HT-2	1~100	y=808x +-250	0.9999	0.4	1.4
T-2	1~100	y=0.00168x 645	0.9998	0.4	1.2
FB1	10~1000	y=350x +232	1.0000	4.8	14.7
FB2	10~1000	y=485x -0.0015	0.9999	5.3	16.0
OTA	1~100	y=0.0018x -387	1.0000	0.4	1.1
ZEA	1~100	y=0.0042x +-5.45	1.0000	0.3	0.9

Forty-seven samples of roasted and ground grains made in Korea were analyzed.

The result on the occurrence of 11 mycotoxins can be seen Table IV. The results showed that the occurrence of zearalenone and deoxynivalenol were frequent. Zearalenone was detected in all samples and deoxynivalenol was detected in 80.9 % samples in the range 0.658 ~ 29.264 μg/kg and N.D ~ 48.332 μg/kg respectively. Fumonisins and ochratoxin A were detected in 46.8% samples and 17 % samples respectively, aflatoxins and T-2/HT-2 toxins were not detected all samples.

TABLE IV
OCCURRENCE OF MYCOTOXINS IN 47 ROASTED AND GROUND GRAIN SAMPLES

	DON (μg/kg)	Afs (μg/kg)	FBs (μg/kg)	OTA (μg/kg)	ZEA (μg/kg)	HT-2 (μg/kg)	T-2 (μg/kg)
Detected Sample No.	38	-	22	8	47	-	-
Conc. Range	0 ~ 48.332	-	0 ~ 66.363	0 ~ 4.125	0.630 ~ 29.264	-	-

REFERENCES

[1] WHO/FAO. Safety Evaluation of certain Mycotoxins in Food, WHO Food Additives Series 47/FAO Food and Nutrition Paper 74, WHO: Geneva, 2001;1

[2] P.Ambrosino, F. Galvano. V., Foglino, A. Logrieco, R. Fresa and A. Ritieni, "Supercritical fluid extraction of Beauvericin from maize",*Talanta*, vol. 62, 2004, pp.523-.530

[3] M.P.E.Gonzalez,J. Mattusch and R. Wennrich, "Stability and determination of aflatoxins by high-performance liquid chromatography with amperometric detection" *J. Chromatogr,A* , vol. 828, 1998, pp. 439-444

[4] L. Gonzalez-Osnaya, C.Cortes, J.M.Soriano, J.C.Molto and J.Manes, "Occurrence of deoxynivalenol and T-2 toxin in bread and pasta commercialized in Spain." *Food chemistry*, vol.124, 2011, pp.156-161.

[5] K.F.Nielsen, U.Thrane, "Fast methods for screening of trichothecenes in fungal cultures using gas chromatography– tandem mass spectrometry" J.Chromatogr. A, vol.929, 2001, pp75-87.

[6] F.Grippi, L.Crosta, G.Aiello, M.Tolomeo, F.Oliveri, N.Gebbia, A.Curione "Determination of stilbenes in Sicilian pistachio by high-performance liquid chromatographic diode array (HPLC-DAD/FLD) and evaluation of eventually mycotoxin contamination." *Food chemistry*, vol.107, 2008, pp.483-488.

[7] R.Krska, R.Schuhmacher, M.Grasserbauer, P.M.Scott, J. "Determination of the Fusarium mycotoxin beauvericin at μ g/kg levels in corn by high-performance liquid chromatography with diode-array detection" *J.Chromatogr. A*, vol. 746, 1996, pp. 233-238

[8] Anne Molinie, Virginie Faucet, Marcel Castegnaro, Annie Pfohl-Leszkowicz. "Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B1: development of a method for simultaneous extraction of ochratoxin A and citrinin." *Food chemistry*, vol. 92, 2005, pp. 391-400

[9] B.Giancaklo, B.Elisabetta, C.Edmondo, C.Valeriana, T.Giuseppina, "Determination of ochratoxin A in eggs and target tissues of experimentally drugged hens using HPLC– FLD." *Food chemistry*, vol. 126, 2011, pp. 1278-1282

[10] S.De Baere, J.Goossens, A.Osselaere, M.Devreese, V.Vandenbroucke, P.De Backer, S.Croubels. "Quantitative determination of T-2 toxin, HT-2 toxin, deoxynivalenol and deepoxy-deoxynivalenol in animal body fluids using LC-MS/MS detection."*J.chromatogr B* vol.879, 2001, pp. 2403-2415.

[11] Z.Zou, Z.He, H.Li, P.Han, J.Tang, C.Xi, Y.Li, L.Zhang, X.Li " Development and application of a method for the analysis of two trichothecenes:Deoxynivalenol and T-2 toxin in meat in China by HPLC-MS/MS" *Meat science*, vol. 90, 2012, pp.613-617.

[12] V. M. T. Lattanzio, M.Solfizzo, S.Powers, A.Visconti. "Simultaneous determination of aflatoxins, ochratoxin A and Fusarium toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity clean up." *Rapid commun. Mass spectrum.*.vol. 21. 2007. pp.3253-3261.

[13] International Conference on Harmonization of Technical Requirements for registration of Pharmaceuticals for Human Use (ICH), Validation of Analytical Procedures Methodology, Geneva, Switzerland, November .1996.