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Article

Fumonisin Measurement from Maize Samples by High-Performance Liquid Chromatography Coupled with Corona Charged Aerosol Detector

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Fumonisins are a class of mycotoxins produced mainly by *Fusarium* species, which is primary fungal contaminant of the maize and maize-derived products around the world. The B-series fumonisins (FB1, FB2 and FB3) are the most abundant and toxic constituent; thus, their levels are regulated generally worldwide. In this study, we developed a reliable method for the measurement of fumonisin FB_1, FB_2 and FB_3 mycotoxins from maize samples without the time-consuming derivatization step using a high-performance liquid chromatograph coupled with corona charged aerosol detector. The detection and quantitation limit of the whole method were 0.02 and 0.04 mg/kg for each fumonisins, respectively. The detection linearity was tested in the calibration range of 2 orders of magnitude and the recoveries from the spiked samples were determined. The developed method proved to be sufficient to measure the maximum residue levels of fumonisins, which are specified in European Union and United States in maize and maize-based products.

Introduction

Fumonisins are group of naturally occurring, polyketide-derived, structurally related mycotoxins produced by *Fusarium* species (1) including *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsushima) (2). However, recently *Aspergillus niger* (3) was also reported as producer of fumonisins. These mycotoxins can cause diseases in animals, including leucoencephalomalacia (4), pulmonary edema (5) and hepatocarcinoma (6). In the case of human effects, the consumption of fumonisin-contaminated products has been associated with high incidences of esophageal cancer in South Africa (7), Italy (8) and Iran (9), as well as primary liver cancer (10). In 2003, fumonisins are considered by International Agency for Research on Cancer to be Group 2B carcinogen to humans (11). Since the first identification of fumonisin B1 (FB1) by Bezuidenhout *et al.* (12), ~100 different fumonisin analogs have been characterized (1, 13–15) and were classified into four main groups, as A-, B-, C- and P-series fumonisins (13). The backbones of A, B and P analogs (FAs, FBs, FPs) contain 20 carbon atoms, whereas the backbone of C-type fumonisins (FCs) consist of 19 carbon atoms. The FBs were reported as the most abundant analogs produced by the wild-type fungal strains with the FB1 making up ~70% of the total fumonisin content. Although, several isolated strains are able to overproduce the FB2 at the level ~80% (16). The order of their toxicities depends on the system being used for the tests, but most frequently FB1 toxin is designated as the most toxic constituent (7).

The Food and Drug Administration in United States has announced guidance levels for sum of FB_1, FB_2 and FB_3 in maize products to protect both human and animal health. The recommended maximum levels of total fumonisins were in the range of 2–4 and 5–100 ppm for human foods and animal feeds, respectively, depending on the type and the proportion of the certain commodities in the total diets (17). The European Union Commission has also recommended guidance levels for fumonisins in feed materials and formulated feedstuffs, which also vary based on different products and ranged from 5 to 60 ppm (18). In the case of human food in the European Union, the maximum residue limits (MRLs) were introduced in 2008 in the range of 0.4–2 ppm (19). The maximum levels of fumonisins are relatively higher in United States than in EU; however, it is important to consider that the later include only the sum of FB_1 and FB_2 mycotoxins.

To meet the challenges of above mentioned MRLs, it is a need sensitive, accurate and reproducible analytical method for the detection of fumonisins in foods and feeds. Furthermore, it is a common requirement for this method that it should be relatively cheap and should contain only minimized sample pretreatment steps to adopt easily in the routine analysis. Several methods for analysis of fumonisins have been developed, but most frequently they are measured by high-performance liquid chromatographic (HPLC) techniques with different detectors including fluorescence (20, 21), evaporative light scattering (ELSD) (22, 23) and mass spectrometer (13, 20). However, the fluorescence detection need additional derivatization steps during the sample preparation, and the procurement and maintenance of mass spectrometric instruments is expensive and requires highly qualified staff. The ELSD detector is relatively cheap and easy to use detection system. In addition, the fumonisins could be detected via ELSD without any derivatization (23), but the complex nature of its response curves sometimes could adversely affect the quantitation including reproducibility and accuracy (i.e., underestimation at lower analyte concentrations and overestimation at higher concentrations). Thus, these factors may be significantly limit the ability to validate and transfer ELSD-based methods among laboratories (24). Besides the generally used HPLC methods, other separation techniques have been also applied and capable to detect and quantify fumonisins such as gas- and thin-layer chromatography (20) and the use of immunological enzyme-linked immunosorbent assay to investigation of the presence of fumonisins in different matrices was also reported (25).

The corona charged aerosol detector (Corona CAD) is also a member of the evaporative detectors such as the ELSD and condensation nucleation light scattering detector (CNLSD) using the following common principles: pneumatic nebulization of the mobile phase containing the analyte eluting from the column to form droplets, and drying of the droplets into particles. However, the way in which analyte mass is determined from particles differs among them. The Corona CAD uses a high-voltage corona needle to charge nitrogen gas than the charged gas collides with analyte particles resulting in the formation of charged particles, which are then collected and measured using a sensitive electrometer (26). In the case of both ELSD and CNLSD use a laser beam and measures the reflected light scattered to a sensitive photomultiplier leading to greater mass results in larger particles, thus greater light scattering (27). According to the literature, Corona CAD provides the broadest dynamic range from the mentioned three type of detectors and shows excellent sensitivity, and uniformity, where the response is independent of chemical structure, while also being the easiest to operate (24).

The Corona-CAD detector has been applied previously for fumonisin detection only in the case of preparative purifications to test the collected fractions after the Centrifugal Partition Chromatographic separation (28), and has not been used so far in quantification of these mycotoxins in agricultural or food products, yet. The purpose of the present study is to develop a reliable HPLC–Corona CAD-based analytical method, which is capable to analyze the level of fumonisins in maize samples.

Experimental

Chemicals and reagents

Methanol, acetonitrile, for sample preparation and eluents were purchased from Biosolve (Netherlands). The formic acid, acetic acid and fumonisin B_1, B_2 standards were obtained from Sigma-Aldrich Ltd. Co. (Budapest, Hungary), while the fumonisin B_3 was gift of Prof. W. C. A. Gelderblom (PROMEC Medical Research Council, Tygerberg, South Africa). Deionized water both for sample preparation and HPLC run was produced by aquaMAX Basic (Young-Lin, Korea) water purification equipment and membrane-filtered water for HPLC runs with a resistivity of 18 MΩ were additionally purified with and aquaMAX Ultra (Young-Lin, Korea) water purification system. TRI-BOX pH-paper was ordered from Macherey-Nagel (Germany). PuriTox TC-F120 columns for the purification fumonisin B_1, B_2 and B_3 mycotoxins were obtained from Trilogy Analytical Laboratory (USA).

Maize samples

Maize samples (at least 1 kg) for the blank and spike samples were taken from the agricultural fields of the Cereal Research Non-Profit Ltd. in the South-Hungary. The samples were delivered at the laboratory immediately after the collection, and tested with HPLC-mass spectrometric method for fumonisin contamination (data not shown).

Standard preparation

An accurately weighed amount (1 mg) of fumonisin B_1, B_2 and B_3 were placed separately in a 5 mL volumetric flask and dissolved in acetonitrile/water, 50/50 (v/v%) (dilution solvent) to produce 200 μg/mL standard stock solutions. A 200 μL aliquot of each stock solution was added into a vial and was diluted with the dilution solvent up to 1 mL to gain concentration of 40 μg/mL of each fumonisins. This mixed stock solution was diluted serially in the following levels using a dilution solution: 20.0, 10.0, 5.0, 2.5, 1.0, 0.5, 0.6, 0.4 (LOQ) and 0.2 μg/mL (LOD). For evaluation of the possible matrix effects on LOD, LOQ and the linearity, the above mentioned levels were also prepared using the extracts of blank maize sample, which were preconditioned as described in the Sample Preparation.

Sample preparation

The maize samples were prepared according to the manufacturer's instructions with some minor modifications. Briefly, 25 g of ground samples were weighed into an Erlenmeyer flasks and 100 mL 50/50 (v/v%) acetonitrile/water were added and they were covered. The flasks were shaked for 1 h on a horizontal shaker and ~10 mL of extracts were filtered into the 12 mL polypropylene tubes (ViaLab Magyarország Kft., Hungary) and their pH were checked and adjusted into the range of 6–9 with 2 N sodium hydroxide. Four milliliters of the extracts were transferred into 30 mL polypropylene tubes (ViaLab Magyarország Kft., Hungary) and 16 mL of 3/1 (v/v%) methanol/water was added to each. Strong anion exchange (SAX) solid-phase extraction (SPE) tubes (PuriTox TC-F120, Trilogy Analytical Laboratory, USA) were conditioned by rinsing with 5 mL of methanol followed by 5 mL of 3/1 (v/v%) methanol/water. The contents of each tube contained the diluted extracts were drained through the columns, which were washed with 16 mL of 3/1 (v/v%) methanol/water and 4 mL of methanol. Finally, the analytes were eluted with 10 mL of 99/1 (v/v%) methanol/acetic acid and evaporated to dryness with stream of nitrogen at 60°C. Before the analytical measurements, the evaporated samples were resolved in 100 μL of 50/50 (v/v%) acetonitrile/water and transferred into HPLC sample vials.

Instrumentation

The measurements were carried out on a YL9100 modular HPLC (Young-Lin, Korea) system equipped with a YL9101 Vacuum degasser, YL9110 Quaternary Gradient Pump, YL9150 Autosampler, YL9130 Column Compartment, which was controlled by YLClarity v. 2.6.5.459 software. Separations were achieved on a YMC-Pack ODS-A 250 × 4.6 mm (YMC, Germany) column with 5 μm particle size, coupled with a YMC ProC18 20 × 4.0 mm (YMC, Germany) guard column, with 5 μm particle size. Eluents A and B were water and acetonitrile, and both of them contained 0.1% (v/v) formic acid. During the run, flow rate was maintained at 1 mL/min, while column temperature was 35°C. To ensure good separation, a gradient program was used as following: starting at 25% B, which was risen to 40% at 22 min, to 100% at 27 min, and this value was held for 3 min, and finally linearly decreased to the starting value for 2 min, and was held until the pressure stabilized. Injection volume was 10 μL.

For the detection, Corona CAD (ESA BioScience, Great-Britain) was applied with an output range of 100 pA, while the nitrogen gas pressure was held on 35 psi, and electronic filter was varied during the optimization. The chromatographic column was connected through an in-line filter (Upchurch, USA) to the detector.

Results

Optimization of detection

For chromatographic separation of fumonisin B1, B2 and B3, the applied gradient elution parameters were based on our earlier works (13); however, the stationary phase was changed to a YMC-Pack ODS-A column (250 × 4.6 mm). The mobile phase contained formic acid to protonate the carboxyl groups attached to the fumonisin backbone in order to the proper retention. The applied eluent was proper for the Corona CAD detector, because it did not contain any non-volatile components and its pH was below of 7.5, which are the requirements for this detection system. Under the established conditions, FB_1 gave a peak at a retention time of 15.32 min, which were 20.50 and 24.14 for FB_3 and FB_2, respectively (Figure 1).

The Corona-CAD detector provides four level for the signal filtering to electronically reduce the noise during the chromatographic run setting the filter time as none, low, medium and high. Using this filtering effects, it is possible to modify the sensitivity of the detection method based on the changes of signal-to-noise (S/N) values. During the optimization period, the effects of all filter time were investigated and no significant differences were observed among the S/N values of each of examined fumonisins (data not shown), thus for the further measurements the raw data were acquired without any filtering.

Linearity

Each of standard fumonisins, ranging from 0.4 to 40 μg/mL (from 4 to 400 ng on column) in both dilution solution and blank sample extract, was tested for their corresponding response as peak areas on the HPLC–Corona CAD. The corresponding levels of two type of dilution showed same area results. The Corona detector response, according to the manufacturer's instructions, has not a linear character and rather a quadratic plot type should be used to describe the response. However, we found that simple concentration–peak area plots were proper for the calibration over the used concentration range, and it is not need to construct the recommended quadratic- or log–log plots such as at ELSD for the calibration curve. The solutions were injected in triplicate and the founded regression equations by plotting the peak areas are in Table I. The determination coefficients (R^2) obtained for the regression lines (Table I) demonstrate the excellent relationship between peak area and the concentrations.

Limit of detection and quantitation

FBs of various concentrations were injected in both dilution solution and blank sample extract into the column to determine the limit of detection. The HPLC–Corona-CAD method was sufficiently sensitive to detect 2 ng each of examined fumonisins per injection, and therefore, the limit of detection for components in solution is ~0.2 μg/mL. The mycotoxin peaks could be detected without any baseline noise disturbances (>3 times) at these concentrations. The limit of quantification was 0.4 μg/mL for each fumonisins, because the analytes' response at these concentration levels were 10 times higher than the baseline noise. The results of LOD and LOQ samples showed no significance differences between the matrix matched and solvent diluted standards.

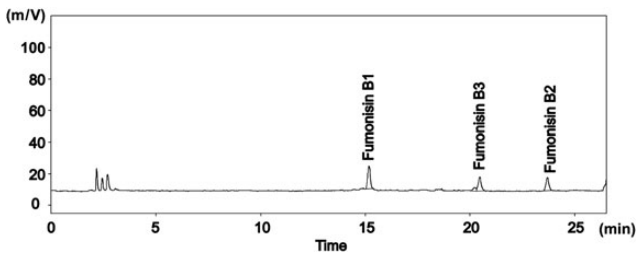

Figure 1. HPLC elution profiles of fumonisin FB1, FB2 and FB3 produced with HPLC–Corona CAD system at the calibration level of 2.5 μg/mL.

Table I
Linearity Results of the Developed HPLC–Corona CAD Method in the Concentration Range of 0.4–40 μg/mL

Components	Equation for regression line	R^2
Fumonisin FB_1	$y = 49.966x + 7.924$	0.9993
Fumonisin FB_2	$y = 40.186x - 10.290$	0.9993
Fumonisin FB_3	$y = 41.14x - 25.165$	0.9973

Precision of chromatographic system

The precision of the chromatographic method, reported as percent relative standard deviation (RSD), was estimated only by measuring repeatability as intraday assay precision, because the calibration will be carried out before every sample series on each day in the routine analysis. This examination is proper to check the measurement suitability of the system, which were tested on six replicate injections at concentration of 10.0 μg/mL of each fumonisins. The RSD values for retention time (min) were 0.35, 0.16 and 0.17% and for peak area were 3.72, 4.90 and 4.78% for FB1, FB2 and FB3, respectively.

Recovery of spiked samples

The repeatability of the whole analytical procedure including sample clean-up and the separation complies with the general analytical requirements, which is affected by selective adsorption of the purification columns or in the case of fluorescence detection by unstable derivatization (29). In our cases, the accuracy and precision of the method were sufficiently high for tested agricultural commodities spiked within the range of 0.3–1.2 μg/g, which represents the low levels of contamination in terms of FB_1, FB_2 and FB_3 occurrence.

The recoveries of fumonisins in this range varied from 82.18 to 91.09%, 82.37 to 92.07% and 82.44 to 89.84% for FB_1, FB_2 and FB_3, respectively (Table II).

Clean-up procedure is an important step in analysis of components from complex matrices mainly using the universal detection. Based on our results, the applied purification step was proper for this purpose, because the chromatographic run of blank maize samples did not contained any interfering peaks at the elution time of the examined fumonisins, and in the case of spiked samples the fumonisin peaks separated properly from the matrix peaks (Figure 2).

Discussion

The retention times of the non-derivatized fumonisins in the achieved separation were similar to the chromatographic

Table II
Recovery of HPLC–CAD Measurements Resulting from Analysis of Fumonisin-Spiked Blank Maize Samples

Spiking level (μg/g)	Recovery (%)	RSD, $n = 5$ (%)
Fumonisin B_1		
0.35	91.09	8.38
0.7	94.95	6.41
1.2	82.18	2.79
Fumonisin B_2		
0.3	88.47	4.65
0.7	82.37	7.84
1.2	92.07	3.41
Fumonisin B_3		
0.3	89.84	2.35
0.7	86.53	3.85
1.2	82.44	4.21

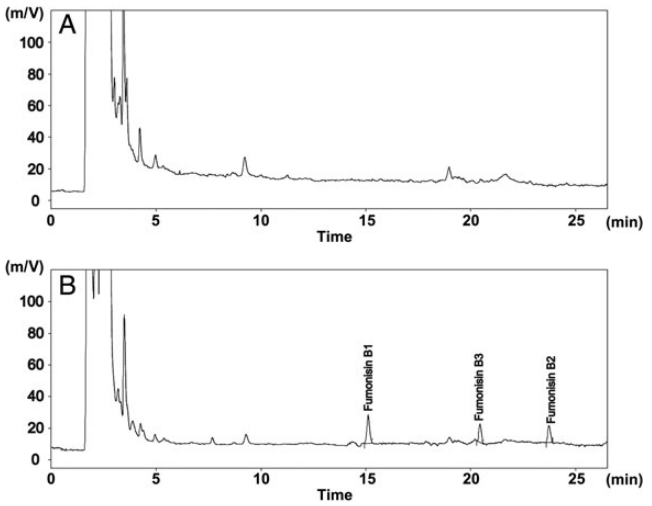

Figure 2. Corona-CAD chromatograms of blank (A) and spiked (B) samples at the level of 0.35 μg/g for FB1 and 0.3 μg/g for FB2 and FB3.

separation developed by Wilkes *et al.* (22) using an ELSD detector and different stationary and mobile phases such as base-deactivated C8 column and acetonitrile–water–trifluoroacetic acid eluent, respectively. However, the resolution among the components were higher than the method reported by Wang *et al.* (23) using also an ELSD for the detection, where the examined fumonisins eluted close to each other in one group of peaks, especially the peaks of FB3 and FB2 were almost overlapping (23). The calibration curve of each examined fumonisin was linear in the used concentration range. According to the literature, it is known that Corona-CAD response is non-linear at a range of 4 orders of magnitude, but its signal is nearly linear or completely linear in the smaller concentration ranges as reported by Błazewicz *et al.* (30) in the case of atracurium, cisatracurium and mivacurium (1–150 μg/mL) and by Grembecka *et al.* (31) for aspartame and caffeine (0.25–75 μg/mL), respectively. The observed sensitivity for FB1, FB2 and FB3 were 30 times higher than in the case of earlier reported ELSD method, where the detection limit was 60 ng per injection for the FB1 (22, 23). However, the detection limit in the case of fluorescent detection of fumonisins was slightly lower than our values, but it used an additional precolumn derivatization reaction with naphthalene-2,3-dicarboxaldehyde (21), which allow to extend the sample pretreatement procedure and to increase the complexity and cost of the analysis. The interfering matrix components in the samples could be removed successfully using the SAX-SPE tubes, because no any matrix effects were observed at the LOD, LOQ and linearity investigations. The recovery rates of FB1 were usually between 74 and 89% in the literature, depending on the type of quantification method (29). In the case of HPLC–ELSD analysis of FB_1, the recovery range was from 77.27 to 102.58% described by Wang *et al.* (23) using Amberlite XAD-4 phase for clean-up procedure. In our study, the recovery values of each fumonisins were in the abovementioned ranges, and their reproducibility proved to be also proper for the routine fumonisin analysis.

Conclusions

This is the first study in which an HPLC–Corona CAD method has been established to separate and determine FB1, FB2 and FB3 mycotoxins from agricultural samples. It is demonstrated that this technique provides an appropriate and reliable alternative to other detection methods for rapid determination of worldwide regulated fumonisin contents without any additional derivatization procedure. The performance of method corresponded well to the analytical requirements and the recoveries of fumonisins were over 82% in all examined cases from maize matrices. The developed method ensures accurate quantification with a limit of 0.4 μg/mL (0.04 ppm), which is sufficiently sensitive for detection of the fumonisin level in extracts of maize and maize products. Based on successful linearity, precision and recovery results of the developed method, it was introduced and applied in the routine analysis of an accredited testing laboratory.

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References

1. Rheeder, J.P., Marasas, W.F.O., Vismer, H.F.; Production of fumonisin analogs by *Fusarium* species; *Applied Environmental Microbiology*, (2002); 68(5): 2101–2105.
2. Seifert, K.A., Aoki, T., Baayen, R.P., Brayford, D., Burgess, L.W., Chulzes, S., *et al.*; The name *Fusarium moniliforme* should no longer be used; *Mycological Research*, (2003); 107: 643–644.
3. Frisvad, J.C., Smedsgaard, J., Samson, R.A., Larsen, T.O., Thrane, U.; Fumonisin B2 production by *Aspergillus niger*; *Journal of Agricultural Food Chemistry*, (2007); 55(23): 9727–9732.

4. Kellerman, T.S., Marasas, W.F.O., Thiel, P.G., Gelderblom, W.C.A., Cawood, M., Coetzer, J.A.W.; Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1; *The Onderstepoort Journal of Veterinary Research*, (1990); 57(4): 269–275.
5. Harrison, L.R., Colvin, B.M., Green, J.T., Newman, L.E., Cole, J.R.; Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*; *Journal of Veterinary Diagnostic Investigation*, (1990); 2(3): 217–221.
6. Gelderblom, W.C., Abel, S., Smuts, C.M.; Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion; *Environmental Health Perspectives*, (2001); 109(S2): 291–300.
7. Marasas, W.F.O.; Discovery and occurrence of the fumonisins: a historical perspective; *Environmental Health Perspectives*, (2001); 109(S2): 239–243.
8. Franceschi, S., Bidoli, E., Baron, A.E., La Vechhia, C.; Maize and the risk of cancers of the oral cavity, pharynx, and esophagus in north-eastern Italy; *Journal of the National Cancer Institute*, (1990); 82(17): 1407–1410.
9. Shephard, G.S., Marasas, W.F.O., Leggott, N.L., Yazdanpanah, H., Rahimian, H., Safavi, N.; Natural occurrence of fumonisins in corn from Iran; *Journal of Agricultural and Food Chemistry*, (2000); 48(5): 1860–1864.
10. Ueno, Y., Lijima, K., Wang, S.D., Sugiura, Y., Sekijima, M., Tanaka, T., *et al.*; Fumonisins as a possible contributory risk factor for primary liver cancer: a 3-year study of corn harvested in Haimen, China, by HPLC and ELISA; *Food and Chemical Toxicology*, (1997); 35(12): 1143–1150.
11. IARC; Fumonisin B1, IARC monographs on the evaluation of carcinogenic risk to humans: some traditional medicines, some mycotoxins, naphthalene and styrene; *IARC Monographs*, (2002); 82: 301–366.
12. Bezuidenhout, S.C., Gelderblom, W.C.A., Gorst-Allman, C.P., Horak, R.M., Marasas, W.F.O., Spiteller, G., *et al.*; Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*; *Journal of the Chemical Society, Chemical Communications*, (1988); 11: 743–745.
13. Bartók, T., Szécsi, Á., Szekeres, A., Mesterházy, Á., Bartók, M.; Detection of new fumonisin mycotoxins and fumonisin-like compounds by reversed-phase high-performance liquid chromatography/electrospray ionization ion trap mass spectrometry; *Rapid Communications in Mass Spectrometry*, (2006); 20(16): 2447–2462.
14. Bartók, T., Tölgyesi, L., Szekeres, A., Varga, M., Bartha, R., Szécsi, Á., *et al.*; Detection and characterization of twenty-eight isomers of fumonisin B1 (FB1) mycotoxin in a solid rice culture infected with *Fusarium verticillioides* by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight and ion trap mass spectrometry; *Rapid Communications in Mass Spectrometry*, (2010); 24(1): 35–42.
15. Gelderblom, W.C.A., Sewram, V., Shephard, G.S., Snijman, P.W., Tenza, K., Van der Westhuizen, L., *et al.*; Structure and natural occurrence of stereoisomers of the fumonisin B series mycotoxins; *Journal of Agricultural and Food Chemistry*, (2007); 55(11): 4388–4394.
16. Szécsi, Á., Szekeres, A., Bartók, T., Oros, G., Bartók, M., Mesterházy, Á.; Fumonisin B(1-4)-producing capacity of Hungarian *Fusarium verticillioides* isolates; *World Mycotoxin Journal*, (2010); 3(1): 67–76.
17. FDA. (2001) Guidance for Industry. Fumonisin Levels in Human Foods and Animal Feeds; Final Guidance. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm109231.htm.
18. 2006/576/EC. Commission of European Communities, Commission recommendation of 17 August, 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding; (2006).
19. 1881/2006/EC. Commission of European Communities, Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs; (2006).
20. Shephard, G.S.; Chromatographic determination of the fumonisin mycotoxins; *Journal of Chromatography A*, (1998); 815(1): 31–39.
21. Lino, C.M., Silva, L.J.G., Pena, A.L.S., Silveira, M.I.; Determination of fumonisins B1 and B2 in Portuguese maize and maize-based samples by HPLC with fluorescence detection; *Analytical and Bioanalytical Chemistry*, (2006); 384(5): 1214–1220.
22. Wilkes, J.G., Sutherland, J.B., Churchwell, M.I., Williams, A.J.; Determination of fumonisins B1, B2, B3 and B4 by high performance liquid chromatography with evaporative light scattering detection; *Journal of Chromatography A*, (1995); 695(2): 319–323.
23. Wang, J., Zhou, Y., Wang, Q.; Analysis of mycotoxin fumonisins in corn products by high-performance liquid chromatography coupled with evaporative light scattering detection; *Food Chemistry*, (2008); 107(2): 970–976.
24. Bailey, B., Plante, M., Crafts, C., Acworth, I.; Advances in Universal Detection, Dionex Corporation, ESA—A Dionex Company; (2010); http://www.dionex.com/en-us/webdocs/87448-PO-HPLC-Universal-Detection-30June2010-LPN2562-01.pdf.
25. Torres, M.R., Sanchis, V., Ramos, A.J.; Occurrence of fumonisins in Spanish beers analyzed by an enzyme linked immunosorbent assay method; *International Journal of Food Microbiology*, (1998); 39(1–2): 139–143.
26. Gamache, P.H., McCarthy, R.S., Freeto, S.M., Asa, D.J., Woodcock, M.J., Laws, K., *et al.*; HPLC analysis of nonvolatile analytes using charged aerosol detection; *LCGC North America*, (2005); 23(2): 150–161.
27. Allgeier, M., Nussbaum, M., Risley, D.; Comparison of an evaporative light scattering detector and a chemiluminescent nitrogen detector for analyzing compounds lacking a sufficient UV chromophore; *LCGC North America*, (2003); 21(4): 376–381.
28. Szekeres, A., Lorántfy, L., Bencsik, O., Kecskeméti, A., Szécsi, Á., Mesterházy, Á., *et al.*; Rapid purification method for fumonisin B1 using centrifugal partition chromatography; *Food Additives and Contaminants Part A*, (2013); 30(1): 147–155.
29. Jackson, L., Jalonski, J.; Fumonisins. In *Mycotoxins in food.* CRC Press, Boca Raton/Boston/New York/Washington, (2004), pp. 380–385.
30. Błazewicz, A., Fijałek, Z., Warowna-Grześkiewicz, M., Jadach, M.; Determination of atracurium, cisatracurium and mivacurium with their impurities in pharmaceutical preparations by liquid chromatography with charged aerosol detection; *Journal of Chromatography A*, (2010); 1217: 1266–1272.
31. Grembecka, M., Szefer, P.; Simultaneous determination of caffeine and aspartame in diet supplements and non-alcoholic beverages using liquid-chromatography coupled to Corona CAD and UV-DAD detectors; *Food Analytical Methods*, (2012); 5: 1010–1017.