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Determination of Fumonisin B_1 and B_2 in Corn Using Matrix-Phase Dispersion Coupled to High Performance Liquid Chromatography

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A simple, rapid and reproducible analytical method for the determination of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in corn samples by matrix solid-phase dispersion procedure coupled to high performance liquid chromatography (HPLC) with photo-diode array detector (DAD). Several dispersants, eluents and ratios were tested during the optimization of the process in order to obtain the best results. Finally, samples were blended with C_{18} and the mycotoxins were extracted with 10 mL of 10 mM formic acid in methanol. Analyses were performed by HPLC-DAD. The mobile phase was a mixture of a 77 % methanol and 23 % 0.1 M NaH_2PO_4 (pH 3.2) solution at 1 mL min^{-1}. The wavelength of detection was 335 nm. The recoveries of the extraction process ranged from 71.0 to 119.7 % with relative standard deviation lower than 15.7 % in all cases, when samples were fortified at three different concentration levels. Limits of detection and quantification were 0.05, 1 and 0.2, 2.0 μg g^{-1}, respectively. Application of the method to the analysis of 13 samples including, corn kernel, flour, corn starch, popcorn, purchased in local supermarkets in Guangzhou city, China, revealed FB_1 and FB_2 levels.

Key Words: Corn, Fumonisins, Matrix solid-phase dispersion, High performance liquid chromatography, Photo-diode array detector.

INTRODUCTION

Fumonisins are constituted as structurally related group of mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum*[1]. Many studies shown that fumonisin B_1 (FB_1), followed by fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) were the most abundant naturally occurring fumonisins in maize[2]. Fumonisins are believed to be responsible for a variety of animal diseases, such as, leukoencephalomalacia in horse, pulmonary oedema in swine and liver cancer in rats[3,4]. Studies on the prevalence of esophageal cancer in regions of South Africa, China, Italy and Iran revealed an association between this disease and the consumption of high fumonisins contaminated maize[3]. Based on their toxicity, fumonisin B_1 has been classified as a potential carcinogen to humans (class 2B)[5]. Corn and corn-based products have been usually reported to be naturally contaminated with fumonisins, particularly FB_1 and FB_2, in every country around the world[2]. As a result of the latter potential risk, the European Commission enforced the limits of 4000 μg/kg of fumonisins (FB_1 and FB_2 sum) for unprocessed maize and 1000 μg/kg for maize intended for direct human consumption[6]. Therefore, it is important to determine FB_1 and FB_2 in maize and corn-based food and feeds in order to evaluate the potential risk for human and animals from contaminated food.

Several methods have been described for the determination of fumonisins in corn and corn products. In most laboratories, mixtures of methanol-water or acetonitrile-water has been the universal extraction solvent for FB_1 and FB_2 and then purification by solid-phase extraction cartridges or immunoaffinity columns[7,8]. However, all these pre-treatments are time-consuming and/or expensive.

Matrix solid-phase dispersion (MSPD) was introduced in 1989, which has many advantages such as simple operation, high recovery rate, less sample and solvent. It is a better choice to traditional extraction methods. At present, MSPD is widely used for the extraction of drugs, pesticides, pollutants and other compounds from the solid and semisolid matrices, such as foods, fruit, vegetable samples or animal tissues[9-15]. However, MSPD is not the conventional methods for analysis of mycotoxins in foods. Its use is limited to few reports of mycotoxins in foods[15].

Due to the lack of a useful chromospheres or fluorophore, direct HPLC to analysis of fumonisins is problematic[16]. The most common analytical method for FB_1 and FB_2 is high-performance liquid chromatography with fluorescence detector (HPLC-FLD) after derivation of *o*-phthaldialdehyde[2], or with mass spectrometry (HPLC/MS)[17]. On the other hand, under certain circumstances, UV detection may provide an alternative to FLD for *o*-phthaldialdehyde derivatives of fumonisins[18].

The aim of this work was developed a new simple and efficient MSPD-HPLC-DAD detection method for the determination of FB_1 and FB_2 in corn and corn-based products and also studied to detect the fumonisins using photo-diode array detection as a practical alternative to the widely used FLD. The extraction method involves the use of a C18 dispersant sorbent. Analytes were eluted with 10 mM formic acid in methanol and extractions were analyzed by HPLC-diode array detection. Finally, the proposed method is successfully applied to the analysis of FB_1 and FB_2 in maize and corn-based products.

EXPERIMENTAL

Methanol, acetonitrile and *o*-phthaldialdehyde (OPA) were supplied by CNW Technologies GmbH (Germany). Solid-phase used for MSPD were octadecysilica (C_{18}) (50 μm) bonded silica, octysilica (C_8) (50 μm), primary secondary amine (PSA) (50 μm) from Dikma Technologies Inc (USA). Florisil (60-100 mesh) was obtained from Sinopharm Chemical Reagent Co. Ltd. (China).

The standards of fumonisin B_1 and B_2 were supplied by Sigma-Aldrich (Madrid, Spain) and Merk (Germany), respectively. All other reagents were of analytical grade. Water for HPLC mobile phase was purified by UNIQUE-R20 purification system with UV+UF optional accessories (Research, China).

The individual stock solutions of fumonisin B_1 and fumonisin B_2 (100 μg/mL) were prepared in acetonitrile:water (1:1, v/v) and stored against light at -20 ºC. The stock solution was diluted to a series of concentrations before use.

Samples: A total of 13 samples were purchased in commercially available samples from supermarkets located in the city of Guangzhou, China. 6 samples of maize-based foods are commercial dry-mills including starch, flour and gluten and the other 7 samples are flaky and granular. The samples were brought to the laboratory with clean plastic bags. The flaky and granular samples were grinded by a laboratory pulverizer, then pass through a 1 mm sieve (> 95 %) and subsequently mixed. All milled samples were stored at -20 ºC until analyzed.

Matrix solid-phase dispersion: 0.5 g of ground samples were weighed and placed into a glass mortar (50 mL) and were gently blended with 0.25 g of C_{18} for 5 min using a pestle, to obtain homogeneous mixture. For the preparation of fortified samples, 50 μL of the standard working solution was added to 0.5 g of sample. Then, they were allowed to stand at room temperature for 2 to 3 h. The homogeneous mixture after solvent evaporated was introduced into a 58 mm × 13 mm i.d. plastic column, which a pieces of PE filter and a layer of absorbent cotton at the bottom and eluted dropwise with 10 mL of 10 mM formic acid in methanol by applying a slight vacuum. Then, extract was transferred to a 10 mL tube and evaporated to dryness at 45 ºC with a gentle stream of nitrogen. The residue was reconstituted to a final volume of 0.5 mL with methanol and filtered through a 13 mm/0.45 μm filter, then stored at 4 ºC until analyzed.

***o*-Phthaldialdehyde derivatization procedure:** The *o*-phthaldialdehyde derivatization procedure used was based on the Shephard *et al*. method[19] with minor modifications. In brief, 50 μL of standards or samples were derivatized with 50 μL of *o*-phthaldialdehyde reagent on the shaker for 30 sec. Then 20 μL was injected for HPLC analysis exactly 3 min after mixed.

Instrumentation: The Agilent 1100 LC system (Agilent, US) consisted of a quaternary pump (G1311A), an on-line membrane system (G1379A) and a photo-diode array detector (DAD) (G1315B). The analytical column was a Agilent TC-C_{18} (150 mm × 4.6 mm, 5 μm) (USA). The column was eluted isocratically at a flow rate of 1 mL/min. Mobile phase was methanol- 0.1 M sodium dihydrogen phosphate (77:23; v/v), which adjusted to pH 3.35 with *o*-phoshporic acid. The detection wavelength was 335 nm. Data were captured on Agilent ChemStation software and quantification was calculated by comparing peak areas with those of authentic fumonisin standards.

RESULTS AND DISCUSSION

Optimization of conditions for matrix solid-phase dispersion: The parameters affecting the MSPD procedure, namely the type and volume of the eluting solvents and the dispersion sorbents, the amount of samples, the pH of eluents, were optimized.

Effect of the solvent on extraction efficiency: The nature of the elution solvent is an important factor for MSPD. According on the previously described method[15], two elution solvents, such as methanol, acetonitrile and three methanol-acetonitrile mixtures (7:3, 5:5, 3:7, v/v), were tested for extraction of FB_1 and FB_2 from maize samples using C_{18} as dispersion sorbent. The results from in Fig. 1 shown, methanol provided the best recoveries for FB_1 and FB_2. The solution of methanol-acetonitrile mixtures or acetonitrile be used as eluent, the recoveries of FB_1 and FB_2 were all declined, FB_2 approximately to zero (acetonitrile as eluent).

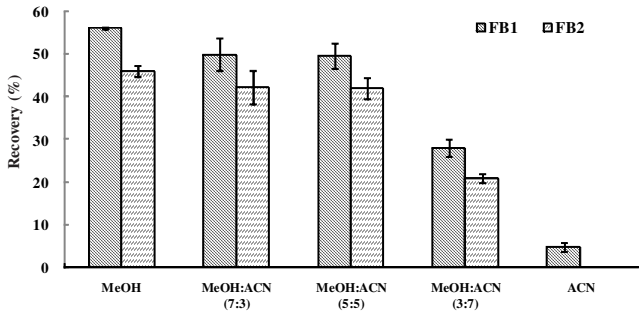

Fig. 1. Recoveries of FB_1 and FB_2 in spiked maize samples with different elution solvents, during the optimization step. C_{18} as dispersion sorbent

To study the effect of pH of elution solvent on extraction, different concentrations of formic acid (1-100 mM) in methanol were tested. The same procedure as it is explained above was carried out: C_{18} was the dispersion sorbent and methanol was the eluting solvent. The result shown the addition of formic acid could improve the extraction of fumonisin B_1 and B_2. The best recoveries (78.9 and 71.0 % for FB_1 and FB_2, respectively) were obtained at the concentration of 10 mM of formic acid (Fig. 2).

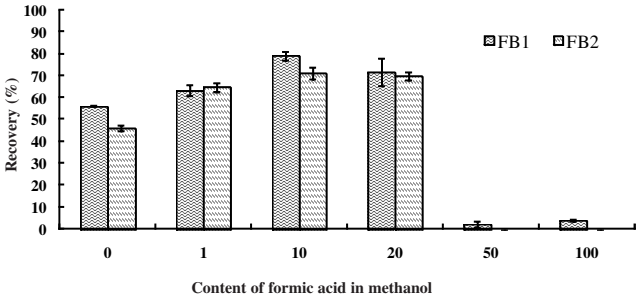

Fig. 2. Recoveries of FB_1 and FB_2 in spiked maize samples with different content of formic acid in methanol, C_{18} as dispersion sorbent

The next step is to determine the volume of eluting solvent, which is necessary to ensure reproducible results for FB_1 and FB_2. So, the effect of eluent volume (4-16 mL) on the recoveries was also tested. The result indicated that the recoveries were the maximum (92.6 and 86.8 % for FB_1 and FB_2, respectively) when the eluting volume was 10 mL. Finally, 10 mL of methanol with 10 mM formic acid was used as eluting solvent (Fig. 3).

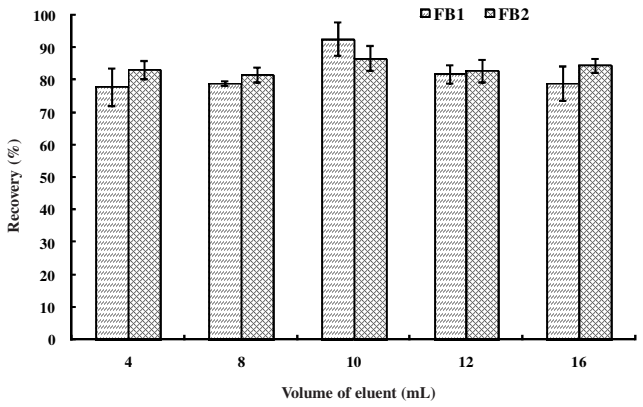

Fig. 3. Recoveries of FB_1 and FB_2 in spiked maize samples with different volume of eluting solutions. 10 mM formic acid in methanol as eluent, C_{18} as dispersion sorbent

Selection of dispersion sorbents: In MSPD, the reversed-phase material, particularly C_{18} and C_8, was the most applicable sorbents. At the same time, Normal-phase, non-bonded sorbents, such as, florisil, amino, phenyl and silica have been also proposed as dispersant in many MSPD applications.

In this study, four different sorbents [C_{18}, C_8, florisil and primary secondary amine (PSA)] were used to investigate their impact on the recoveries of FB_1 and FB_2. As can be seen in Table-1, the best results were obtained when C_{18} was used as dispersant, the recoveries for FB_1 and FB_2 were 92.6 and 86.7 %, respectively. However, the other two phase materials (florisil and PSA) were not feasible for fumonisins as sorbents in MSPD (recoveries were zero). On the other hand, the difference between the mean recoveries obtained with C_{18} and C_8 has no statistical significance. The C_{18} was chosen as the optimum dispersion sorbent due to their hydrophobic characteristics which provided high affinity for FB_1 and FB_2.

The ratio of dispersing material to matrix is another critical parameter in MSPD. Different amounts of C_{18} were added the maize sample (0.5 g) in the glass mortar and then elution was performed with 10 mL of 10 mM formic acid in methanol.

TABLE-1
INFLUENCE OF DIFFERENT SORBENTS AS DISPERSANT ON THE RECOVERY OF FB_1 AND FB_2 IN SPIKED MAIZE BY MSPD EXTRACTION

Dispersion adsorbents	FB_1		FB_2	
	Recovery	RSD (%)	Recovery	RSD (%)
C18	92.6	8.9	86.8	6.5
C8	64.2	2.6	78.3	2.4
PSA	0	0	0	0
Florisil	0	0	0	0

"0" means fumonisins no be detected in the spiked samples after MSPD extraction with PSA and florisil as dispersant. Data for triplicate extraction.

The results showed that the ratio have no obviously effect on the recoveries of FB_1 and FB_2. When the range of ratio was between 3:1 and 1:4, recoveries were in acceptable range of 70.8-98.8 %. In order to minimize the use of inorganic material sorbent, 1:2 ratio (0.25 g of C_{18} and 0.5 g of corn sample) was selected for this study (Fig. 4).

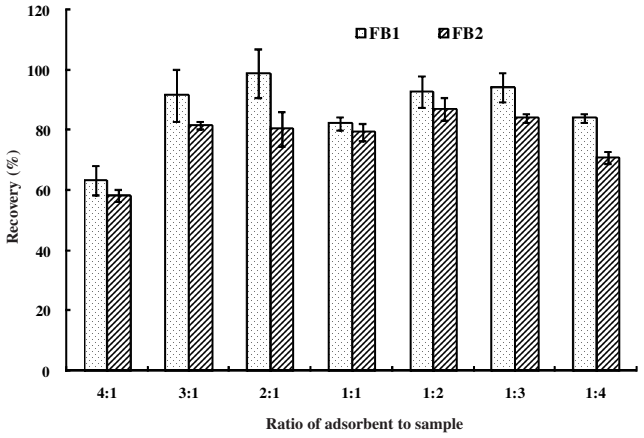

Fig. 4. Recoveries of FB_1 and FB_2 in spiked maize samples with the ratio of sorbent to sample. C_{18} as dispersion sorbent

Analytical characteristics of the method: The method was validated for linearity, detection and quantification limits, selectivity, accuracy and precision. Calibration curves were prepared at five levels. Each concentration was injected for triplicate. For fumonisin B_1 (FB_1), the regression equation was $y = 5.2854x + 0.4320$, with regression coefficient (r^2) of 0.9999. For fumonisin B_2 (FB_2), the regression equation was $y = 1.1808x - 0.4437$, with regression coefficient (r^2) of 0.9987. The limit of detection (LOD) (S/N 3:1) was 0.05 µg/g and 1µg/g and the limit of quantification (LOQ) (S/N 10:1) was 0.2 and 2.0 µg/g for FB_1 and FB_2, respectively.

Recoveries of the MSPD extraction method were carried out by spiking blank maize samples with known volumes of the appropriate working standard solution (3 spiked levels). The average recoveries of FB_1 and FB_2 were listed Table-2. The result shown that the recoveries were in the range of 71.0-119.7 %, with a relative standard deviation (RSD) less than 15.7 %. According to the EU criteria (an average recovery between 70 and 120 % and RSD lower than 20 %) (EC, 2002), the method was considered "acceptable".

Application of the method to real samples: The proposed method was applied to the analysis of 13 real maize samples, including in corn kernels, flour, corn starch, corn soup, flakes

TABLE-2
RECOVERY AND PRECISION RESULT OF STANDARD ADDITION IN MAIZE SAMPLES (n = 5)

Spiked level (mg/kg)	FB_1		Spiked level (mg/kg)	FB_2	
	Average of recovery (%)	RSD (%)		Average of recovery (%)	RSD (%)
0.2	119.7	15.7	2	85.3	11.1
0.5	94.2	9.8	5	73.4	5.2
1.0	71.0	9.2	10	89.2	3.9

and popcorn. The results from the real samples show that only fumonisin B_1 was detected in five samples, the concentration of FB_1 was the range of 0.21-0.60 mg/kg. Fig. 5 depicts typical LC chromatograms obtains for one of these samples and standard solution. All of positive samples didn't exceed the fumonisin maximal tolerable levels recommended by the EC (4000 μg/kg for unprocessed maize and 1000 μg/kg for maize intended for direct human consumption) (EC 2006). No samples were found to be contaminated with FB_2 (below the LOQ levels). One reason was that the UV is not more sensitive than fluorescence detection. However, DAD detection is viable for the determination of FB_1, the major fumonisin analogue, at levels of contamination not below 0.2 mg/kg. In fact, the concentration of FB_2 was far lower than the FB_1 in all real samples.

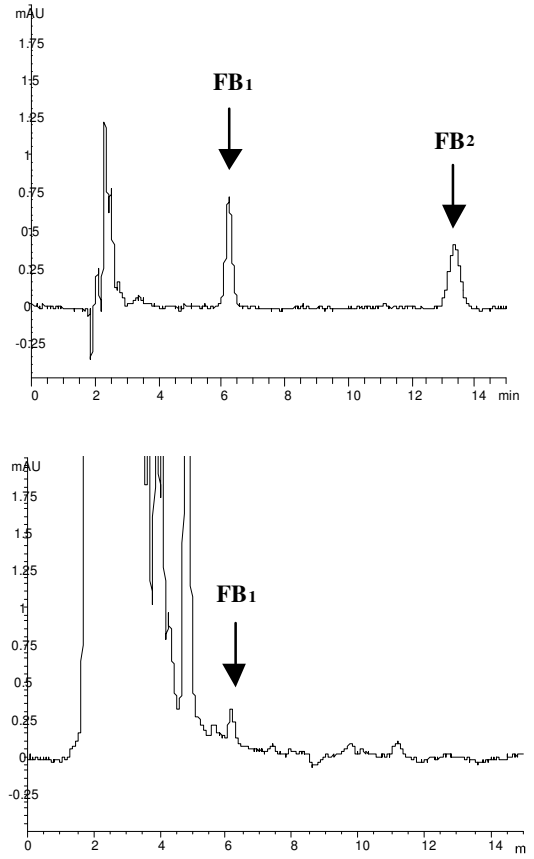

Fig. 5. HPLC Chromatograms of standard solution and real sample. Chromatographic conditions were as explained in section 2. Right is real sample; left is standard solution

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REFERENCES

1. P.G. Thiel, W.F.O. Warasas, E.W. Sydenham, G.S. Shephard, W.C.A. Gelderblom and J.J. Nieuwenhuis, *Appl. Environ. Microbiol.*, **57**, 1089 (1991).
2. G.S. Shephard, P.G. Thiel, S. Stochenstrom and E.W. Sydenham, *J. AOAC Int.*, **79**, 671 (1996).
3. E.W. Sydenham, G.S. Shephard, P.G. Thiel, S. Stockenstrom, P.W. Snijman and D.J. Van Schalkwyk, *J. AOAC Int.*, **79**, 688 (1996).
4. A. Desjardins, Fusarium Mycotoxins: Chemistry, Genetics and Biology: APS Press: St. Paul, MN, p. 79 (2006).
5. International Agency for Research on Cancer (IARC), Fumonisin B_1. In IARC Monogrpahy on the Evaluation of Carcinogenic Risk to Humans, Some Tradditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene; IARC: Lyon, France, Vol. 82, pp. 301-366 (2002).
6. EU, Commission Directive, 1126/2007 EC, Amending Regulation (EC) No, 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Fusarium Toxins in Maize and Maize Products (2007).
7. A. Visconti, M. Solfrizzo and A. De Girolamo, *J. AOAC Int.*, **84**, 1828 (2001).
8. Y.P. Ren, Y. Zhang, S.Y. Han, Z. Han and Y.N. Wu, *Anal. Chim. Acta*, **692**, 138 (2011).
9. J. Blesa, J.M. Soriano, J.C. Molto, R. Marin and J. Manes, *J. Chromatogr. A*, **1011**, 49 (2003).
10. Y.Y. Hu, P. Zheng, Z.X. Zhang and Y.Z. He, *J. Agric. Food Chem.*, **54**, 4126 (2006).
11. C. Cavaliere, P. Foglia, C. Guarino, M. Nazzari, R. Samperi and A. Lagana, *Anal. Chim. Acta*, **596**, 141 (2007).
12. A. Gentili, F. Caretti, G. D'Ascenzo, L.M. Rocca, S. Marchese, S. Materazzi and D. Perret, *Chromatographia*, **66**, 669 (2007).
13. A. Bacaloni, C. Cavaliere, F. Cucci, P. Foglia, R. Samperi and A. Lagana, *J. Chromatogr. A*, **1179**, 182 (2008).
14. R.N. Wu, F.L. Han, J. Shang, H. Hu and L. Han, *Eur. Food Res. Technol.*, **228**, 1009 (2009).
15. J. Rubert, C. Soler and J. Manes, *Talanta*, **85**, 206 (2011).
16. G.S. Shephard, *J. Chromatogr. A*, **815**, 31 (1998).
17. L. Silva, M.F. Franzon, G. Font, A. Pena, I. Silveira, C. Lino and J. Manes, *Food Chem*., **112**, 1031 (2009).
18. N. Nduble, L. van Der Westhuizen, I.R. Green and G.S. Shephard, *J. Chromatogr. B*, **879**, 2239 (2011).
19. G.S. Shephard, E.W. Sydenham, P.G. Thiel and W.C.A. Gelderblom, *J. Liq. Chromatogr. Rel. Technol.*, **13**, 2077 (1990).