Analysis of fumonisin, FB1 and FB2 mycotoxins in corn food and feed samples using the Agilent 1120 Compact LC System coupled to the Agilent 6140 Single Quadrupole LC/MS System

Application Note

Food Safety

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Abstract

A simple extraction method (EM) and an immunoaffinity column (IAC) extraction method are compared to analyze fumonisin FB1 and FB2 in food and feed samples. Analysis was performed on an Agilent 1120 Compact LC coupled to an Agilent 6140 Single Quadrupole LC/MS System. Many compounds from the matrix are extracted but may elute at different retention times. The diverter valve in the mass spectrometer effectively eliminates background matrix compounds. The chromatographic method is linear with $R^2 > 0.9999$ (R^2) and has acceptable retention time RSD of < 0.1%. The recoveries of the simple extraction method are higher than that from the IAC method. The recoveries from EM range from 62% to 78% while those from the IAC method range from 42% to 64%. Both methods can successfully analyze food and feed samples. The EM method is more cost effective, while the IAC method has less matrix effects.

Agilent 1220 Infinity LC Verified

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Introduction

Fumonisins are fungal metabolites produced by several Fusarium species.[1] Ingestion of food contaminated with mycotoxins can cause mycotoxicosis, which is an acute or chronic toxicity. The types of fumonisins include FB1, FB2 and FB3, which are known to contaminate corn samples. The European Commission regulates levels of fumonisin in corn and corn-based foods for a total content of FB1 and FB2 in the range of 200–4000 µg/kg depending on the intended use. The U.S. Food and Drug Administration specifies significantly higher limits of all three total fumonisins (FB1+FB2+FB3): a limit of 2000–4000 µg/kg in human food and 5000–100000 µg/kg in animal feed. The AOAC official method 995.15 for FB1, FB2 and FB3 uses a strong anion based sample cleanup followed by LC based analysis and fluorescence detection. Immunoaffinity column use for mycotoxins has increased because of its simple cleanup procedure and lower detection level of mycotoxins. Additionally, simple extraction procedures including Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) or modified QuEChERS methods coupled to LC/MS or LC/MS/MS have been increasingly used as alternatives to traditional derivatization and extensive cleanup. In this Application Note, fumonisin is extracted from corn samples and corn-based food and feed samples using both the immunoaffinity columns and simple extraction procedure. The analysis is performed using an Agilent 1120 Compact LC System coupled to an Agilent 6140 Single Quadrupole LC/MS System. The fumonisin metabolite structures FB1 and FB2 are shown in Figure 1. The 722 and 723 are the molecular weights (M+H) of FB1 and the naturally occurring isotope (A+1). The 706 and 707 are the molecular weights (M+H) of FB2 and its isotope (A+1). The ratio of the molecular weights to that of its isotope is 2.5 for both mycotoxins.

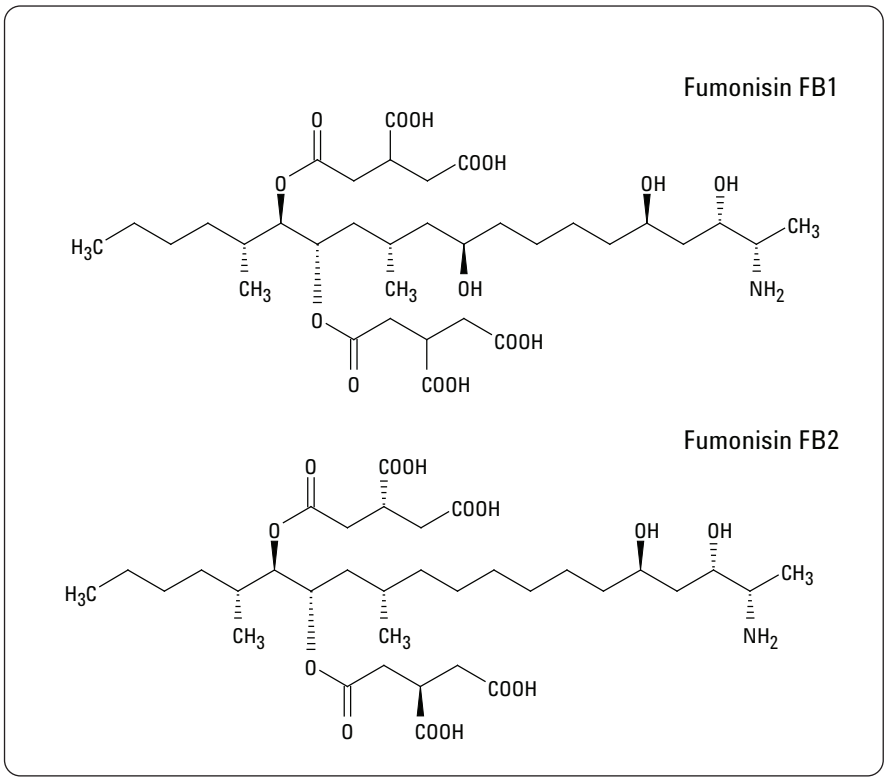

Figure 1
Shows the structure of fumonisin FB1 and FB2 metabolite structure.

The major challenge for mycotoxin analysis in food is that different extraction procedures may be required for different food matrices. It is esseential to optimize the extraction procedure by performing recovery and matrix effect experiments at different concentrations. Immunoaffinity columns (IAC) have the advantage of generating less of a matrix effect but the procedure is expensive. Many simple extraction procedures are available that can potentially extract mycotoxins in one extraction step[2]. QuEChERS includes two steps; an extraction step followed by a cleanup step. Only the extraction step is used in the EM thereafter the samples are filtered by a 0.2 µ filter. To keep the mass spectrometer clean, the diverter valve is opened at a specific time to allow only the analytes of interest to enter the mass spectrometer.

Experimental

The fumonisin mixture (FB1 and FB2, 50 ug/mL, 99% pure) was purchased from Sigma Aldrich. The mobile phase modifiers used were of LC/MS grade. Methanol was LC/MS grade from Sigma Aldrich. Acetonitrile was SuperGradient from Labscan and water used was Milli-q system from Millipore. Phosphate buffer saline (PBS) pH was made by dissolving 8.9 g of disodium hydrogen phosphate dehydrate, 8 g of sodium chloride and 0.2 g of potassium chloride in 1 L water. The pH was adjusted with orthophosphoric acid to 7.4.

Blank corn samples

Blank samples had no fumonisin present. Blank corn matrix was determined by extracting fumonisin from various corn samples obtained from local markets in India. The fumonisin levels were determined both by EM and IAC methods. Blank corn samples included locally found corn flakes and a type of corn in cans.

Fumonisin linearity solutions

Linearity solutions were prepared[1]. Working standard solutions of 8 µg/mL were made from 50 µg/mL stock solutions by diluting with methanol. Calibration stock solution of 1 µg/mL was made from working standard solutions by diluting 50:50 acetonitrile: water. Working calibration solutions (4 ng/mL, 8 ng/mL, 15 ng/mL, 27 ng/mL, 63 ng/mL, 88 ng/mL, 128 ng/mL, 168 ng/mL, and 220 ng/mL) were made from calibration stock solution by diluting 50:50 methanol:water. Linearity curves were generated from working calibration solutions on three different days. Linearity samples were run before each sample analysis.

Experimental Parameters	Details
Column	Agilent ZORBAX Eclipse Plus XDB-C8, 30 mm × 100 mm, 3.5 µm p/n 961967-306; operated at 40 °C
Mobile phase	Buffer A: 0.005 M ammonium acetate buffer pH 3.1 (0.385 g of ammonium acetate in 1.00L of water, pH adjusted with 16 mL acetic acid) Buffer B: 100% methanol
Gradient run	Run Time (min): 17 min 0 min – 50% B (diverter valve switched to waste) 2 min – 50% B (at 5 min divertor valve switched to MS) 4 min – 65% B (at 10 min divertor valve switched to waste) 17 min - 95% B Post time: 10 min
Flow	0.5 mL/min
Injection volume	20 µL, needle wash using 50% method-50% water
Agilent 6140 LC/MS parameters	
SIM mode	706, 707, 722, 723 in time segment of 5 to 10 min
ESI Source	Positive mode
Dwell time	208 msec[1]
Drying gas	10.0 L/min
Nebulizer pressure	50 psig
Dry gas temperature	350 °C
Capillary Voltage (+)	3500 V
Fragmentor	150 V

System suitability test

System suitability tests were performed[1]. In this study a fumonisin mixture made with 10 ng/mL each FB1 and FB2 was injected 10 times to determine if the RSD of retention time (RT) was less than 10%. Ten injections of fumonisin mixture at a concentration of 650 ng/mL each of FB1 and FB2 were made to determine if the area RSD was less than 5%. The isotope ion ratio for FB1 722/723 and for FB2 706/707 was in the range of 2.5 ± 0.2.

Filter test

About 0.5 ppm standard fumonisin mixture prepared in 50% methanol and 50% water was subjected to three injections before and after filtration with respective filters: PVDF syringe filter (Millipore, 0.22 µm), Nylon syringe filter (Millipore, 0.45 µm), regenerated cellulose syringe filter (Agilent, 0.2 µm) and cellulose acetate spin filter (Agilent, 0.22 µm). The percentage recovery was determined from the area percentage.

Samples extraction procedure using IAC

The IAC for fumonisin was purchased from Romer Labs (Fumoni Star, COIAC 3000). The sample extraction procedure was used per the manufacturer with some modifications[1]. Twenty-five milliliters of extraction buffer – acetonitrile/methanol/water (25/25/50) were added to 10 g of crushed sample. Additionally, 1 g of sodium chloride was added. The samples were kept on an orbital shaker (VWR Mini Shaker, speed of 800 rpm) for 20 min. The samples were then centrifuged at 2500 g for 10 min, and the supernatant collected. The process was repeated with an additional 25 mL of extraction buffer and the supernatant was pooled. The pooled sample was centrifuged or filtered with an Agilent regenerated cellulose filter (p/n 5061-3361) and 10 mL

were mixed with 40 mL of PBS to obtain a 50 mL solution. This mixture was centrifuged or filtered with an Agilent regenerated cellulose filter and 10 mL from a 50 mL solution were added to the IAC fitted to an Agilent 20-port vacuum manifold assembly (p/n 5982-9120). The solution was allowed to flow through an IAC by gravity. Subsequently, 10 mL of PBS was passed through the IAC for cleanup. Without letting the IAC dry, 3 mL of methanol containing 2% acetic acid were added followed by another 3 mL of water to collect fumonisin. This extract was subjected to LC/MS.

Sample extraction procedure using EM

One hundred milligrams of $MgSO_4$, 50 mg of Sodium Chloride and 1 mL extraction buffer were added to 200 mg of samples that were crushed using 600 W mixer. The samples were kept on an orbital shaker for 40 min then centrifuged at 3000 g for 10 min. The supernatant was collected. Another 1 mL of extraction buffer was added and the sample kept on an orbital shaker for 20 min and centrifuged again. The pooled supernatant was filtered through an Agilent cellulose acetate spin filter (p/n 5185-5990). To 0.5 mL of the filtrate, 0.75 mL of water were added followed by a second spin filter step. The second filtrate was subjected to LC/MS.

Recovery studies

Blank corn samples were used for the recovery experiment. Samples for the IAC method (10 g) and the EM method (200 mg) were spiked with the appropriate concentration of fumonisin mixtures to obtain final concentrations of 200 ng/g and 800 ng/g for each method. Three samples were run on two different days.

Matrix effect

To determine the matrix effect, blank corn samples were extracted by the IAC method and EM. In the IAC method, 1 mL of the blank corn sample extract was spiked with 53 ng of fumonisin standard. For the aqueous sample comparison for the IAC method, the IAC elution buffer was used. A 0.5 mL amount of methanol containing 2% acetic acid was mixed with 0.5 mL water to which 53 ng of fumonisin standard was spiked. Similarly for the EM method, 40 ng of fumonisin standard was spiked into 1.25 mL of the blank corn sample extracted by EM method. For aqueous sample comparison, 40 ng of fumonisin were spiked to a mixture of 0.5 mL of extraction buffer and 0.75 mL water. In both methods the final concentration values were equivalent to 800 ng/g concentration in the sample. In order to test the baseline, blank extracts from the respective extraction methods were used without spiking any standards.

Results and discussion

System suitability test

The performance results of the system suitability tests are shown in Table 1. The system suitability results are in accordance with reference 1 and the results within acceptable passing criteria.

The total ion chromatogram of the standard fumonisin mixture is shown in Figure 2. The diverter valve allows eluent from 5 to 10 min into the MS, thereby keeping it clean.

System suitability tests	FB1	FB2	Acceptance criteria
10 ng/mL (n=10)	RSD RT: 0.05%	RSD RT: 0.04%	RSD RT: 10%
650 ng/mL (n=10)	RSD area: 3.7%	RSD area: 3.9%	RSD area: 5%
Ion ratio	722/723: 2.6	706/707: 2.6	2.5 ± 0.2

Table 1
System suitability acceptance criteria for the two fumonisin species are shown.

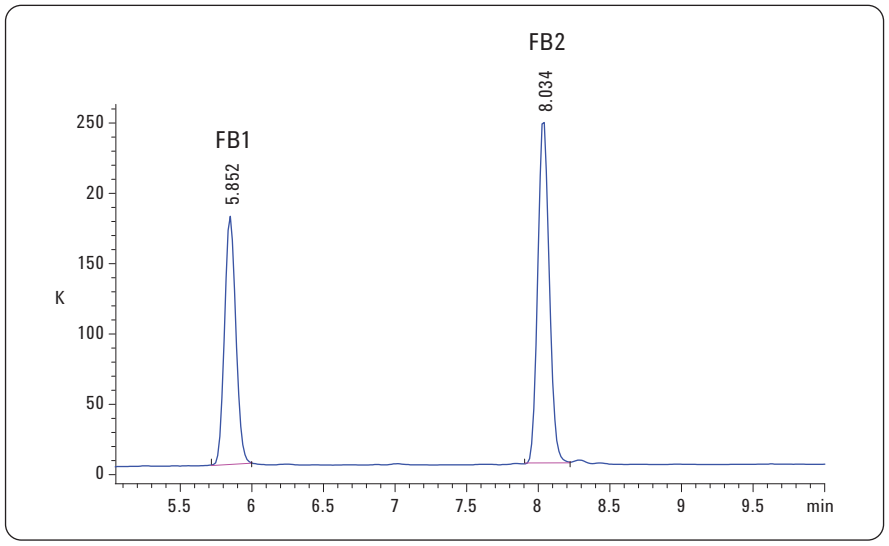

Figure 2
Single ion monitoring (SIM) mode data acquisition of the fumonisin mixture FB1 and FB2.

Filter test

The average percentage recovery results from the filter tests are shown in Table 2. The results show that cellulose acetate, regenerated cellulose and PVDF filters are ideal for fumonisin experiments while nitrile filters are not compatible.

Linearity tests

Linearity samples ranged from 4 ng/mL to 220 ng/mL, (80 picogram to 4.4 nanogram on-column) and were measured on three different days. A linearity > 0.9999 was observed on all three days before all sample runs. The limit of detection (LOD) was 40 pg on-column having a S/N ratio greater than 3.0; while the limit of quantitation (LOQ) was 80 pg on-column having a S/N ratio greater than 10.0.

Recovery studies

Recovery studies were performed with two different concentrations on two different days. The average recovery percentage of FB1 and FB2 are listed in Table 3. The results of IAC are on the low side < 65%. Previous work by Senyuva[1] showed recoveries ranging from 70–138% for an interlaboratory study. The results from the EM method were comparatively higher with recoveries ranging from 62–78%.

Matrix effect

The matrix effect was tested by spiking a known amount of standard in blank corn matrix extract and the same amount in corn buffer. The area of the target analyte determined in the spiked blank matrix is compared against the area of the analyte spiked buffer sample (Table 4). The results show that IAC columns have less matrix effect compared to those with EM method. The value of the EM method is from 90–108% while IAC is from 96–98%.

	PVDF	Nitrile	Regenerated cellulose	Cellulose acetate
FB1	99.98817	59.56	101.82	100.74
FB2	98.21616	45.09	100.35	97.99

Table 2
Average percentage recovery of FB1 and FB2 from various filter types is shown.

Spiked amount of FB1 and FB2	Recovery (%) of FB1	Recovery (%) of FB2
200 ng/g - IAC	46	62
800 ng/g - IAC	42	64
200 ng/g - EM	62	69
800 ng/g - EM	69	78

Table 3
Average recovery percentage of FB1 and FB2 at two different concentrations.

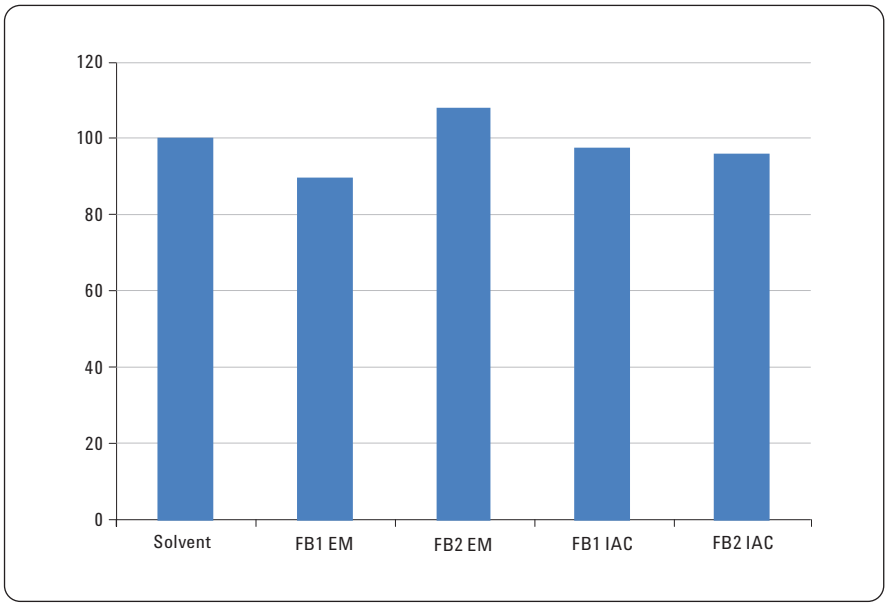

Table 4
Recovery results of FB1 and FB2 each from two different extraction procedures – EM and IAC.

The contribution of the blank matrix to the overall response from both the methods is shown in Figure 3. The results show a cleaner chromatogram from the IAC extraction method.

Fumonisin analysis from food and feed samples

Fumonisin was extracted from human food and animal feed samples using the IAC and EM extraction methods and analyzed on the Agilent 1120 Compact LC coupled to an Agilent 6140 Single Quadrupole LC/MS System. The food and feed samples were purchased from local stores in India, and some were imported food items. Fumonisin results for FB1 and FB2 are corrected for recoveries and the results are shown in Table 5. According to the EU commission 200 µg/kg is the limit for certain types of food. The results show two of the human food items exceeding the EU limit but not crossing the U.S FDA limit. The experimental results from both extraction methods were similar.

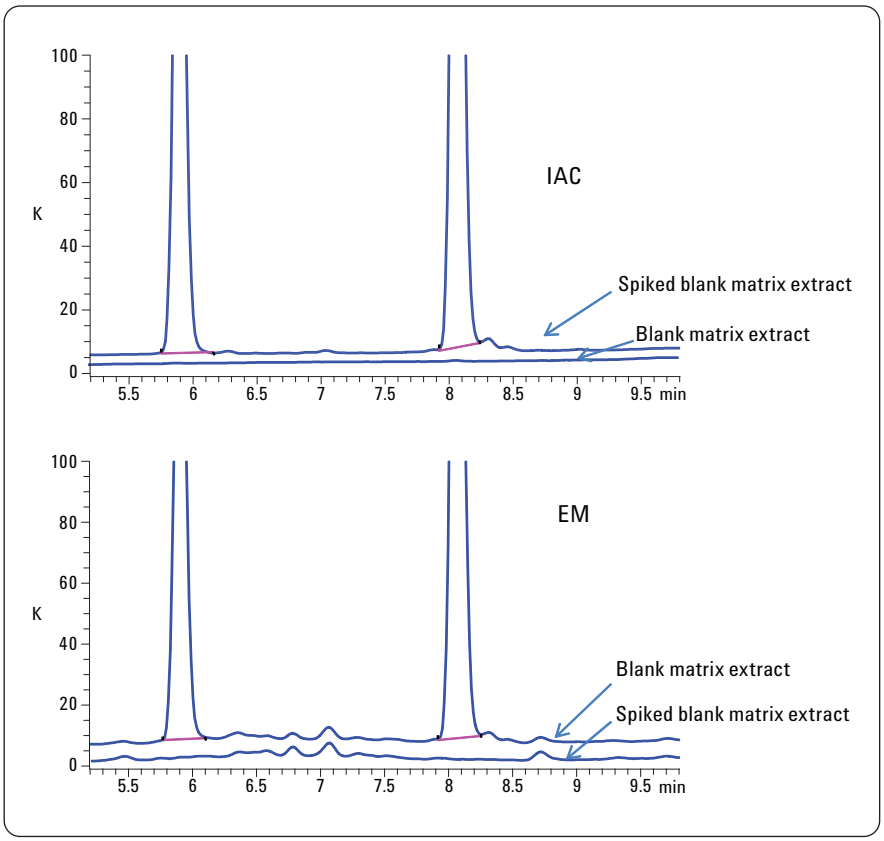

Figure 3
Overlay of blank matrix peaks and the standard spiked in blank matrix peak.

Sample Name	FB1 (µg/Kg)	FB2 (µg/Kg)	Total Fumonisin (µg/Kg)
Dried Corn - IAC	797	204	1001
Dried Corn – EM	735	189	924
Popcorn – IAC	380	139	519
Popcorn – EM	319	123	442
Corn sour/turtle food/rice grain - IAC	ND	ND	ND
Corn sour/turtle food/rice grain - EM	ND	ND	ND

Table 5
Fumonisin analysis on food and feed samples extracted by IAC and EM methods.

Conclusion

Two extraction methods were tested for determining the total fumonisin content in food and feed samples. The results show the recoveries to be better with the EM method than those from the IAC method. However, the matrix effect was less pronounced with the IAC method. The cost of sample analysis is higher in IAC than EM. Comparable results were obtained from both the extraction methods to detect fumonisin in human food samples. The Agilent 1120 Compact LC coupled to an Agilent 6140 Single Quadrupole LC/MS System was found to be a robust instrument for analyzing large numbers of food matrix samples.

References

1.
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2.
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