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CATALYST FOR SUCCESS

ANALYSIS OF FUMONISINS IN GRAINS AND FEED

Fumonisins are naturally occurring Mycotoxins produced by Fusarium mold species. Fumonisins contamination happens worldwide in many agricultural commodities, especially in corn, and is usually associated with dry and hot weather followed by periods of high humidity. Out of more than ten types of Fumonisins, Fumonisin FB1 is the most prevalent and toxic, followed by Fumonisins FB2 and FB3. Fumonisins are classified as possibly carcinogenic for humans and also cause health problems in animals, especially in equids and swine. FDA sets total Fumonisins limits in human foods between 2-4 ug/g and in animal feed between 5-100 ug/g.

Since Fumonisins don't have a chromophore and don't fluoresce derivatization is needed to achieve the required sensitivity of detection. We developed a fast and sensitive HPLC method with post-column derivatization that is capable of analyzing Fumonisins in grains and animal feed at levels as low as 0.01 ug/g.

METHOD

Analytical Conditions

Column: Mycotox reversed-phase C_{18}, 4.6 x 250 mm (Part No: 1612124)

Temperature: 40 °C

Flow Rate: 0.8 mL/min

Mobile Phase: Eluant A: Dilute 1 mL of formic acid to 1 L with D.I. water

Eluant B: MeOH

Injection Volume: 10–50 µL

Post-column Conditions

Post-column System: Pinnacle PCX

Heated Reactor Volume: 1.4 mL

Temperature: 65 °C

Reagent: 950 mL GA 104, 300 mgs OPA, 2 g Thiofluor, 3 mL of 30% Brij 35 solution

Detection: FLD, λ_{ex} = 335 nm, λ_{em} = 440 nm

HPLC GRADIENT		
TIME (Min)	Eluent A, %	Eluent B, %
0	45	55
2	45	55
9	30	70
14	10	90
16	10	90
16.1	45	55
22	45	55

SAMPLE EXTRACTION AND CLEAN-UP

Immunoaffinity clean-up columns: Fumonitest™ WB (Vicam)

Extraction Solution: Water/Methanol (20/80)

PBS Solution: Dilute 100 mL of 10X PBS (Vicam, P/N G1113) to 1 L with DI water

To 25 g of finely ground sample add 2.5 g of NaCl and 50 mL of extraction solution. Blend at high speed for 5 min, filter through fluted filter. Take 10 mL of extract and add 40 mL of PBS solution, mix well, filter through microfiber filter. Load 10 mL of diluted extract to Immunoaffinity column, let the solution pass through at the flow rate about 1-2 drops/sec. Wash the column with 10 mL of PBS solution, elute with 1 mL of Methanol followed by 1 mL of DI water. Evaporate the solution to dryness under the stream of Nitrogen, reconstitute in 1 mL of Methanol/water (50/50). Inject 10–50 uL.

ANALYSIS OF FUMONISINS						
Sample	FB1 found in sample	FB2 found in sample	FB1 spike concentration	FB2 spike concentration	FB1 recoveries	FB2 recoveries
Barley	0 ug/g	0 ug/g	0.2 ug/g	0.07 ug/g	92.3%	87.5%
Milo	0.04 ug/g	0.01 ug/g	0.2 ug/g	0.07 ug/g	85.4%	81.8%
Safflower seeds	0 ug/g	0 ug/g	0.2 ug/g	0.07 ug/g	92.0%	80.8%
Corn	0.17 ug/g	0.03 ug/g	0.4 ug/g	0.13 ug/g	91.7%	88.7%
Oats	0 ug/g	0 ug/g	0.2 ug/g	0.07 ug/g	91.5%	83.3%
Mixed feed	0.08 ug/g	0.02 ug/g	0.3 ug/g	0.1 ug/g	88.0%	80.6%

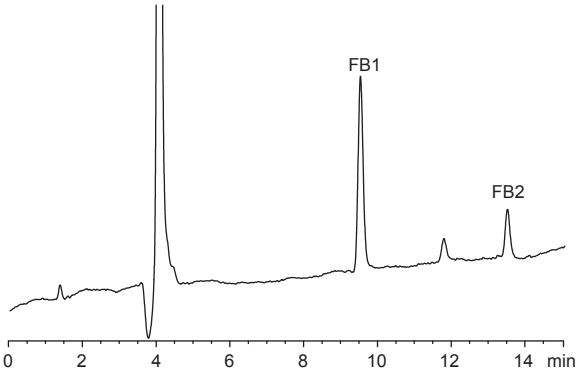

Fig 1. Chromatogram of corn sample contaminated with 0.17 ug/g of FB1 and 0.03 ug/g of FB2

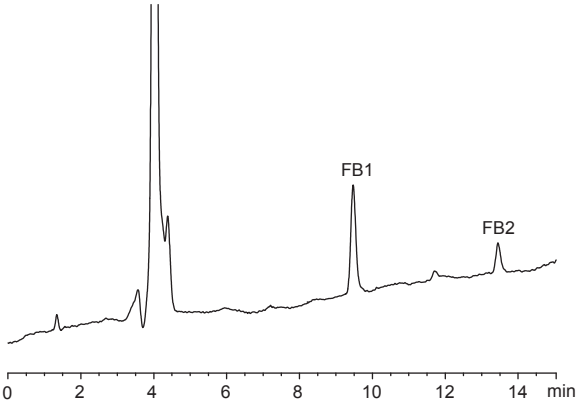

Fig 2. Chromatogram of mixed feed sample contaminated with 0.08 ug/g of FB1 and 0.02 ug/g of FB2

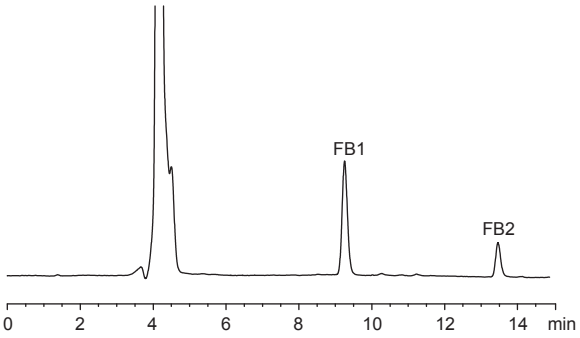

Fig 3. Chromatogram of barley sample spiked with 0.2 ug/g of FB1 and 0.07 ug/g of FB2

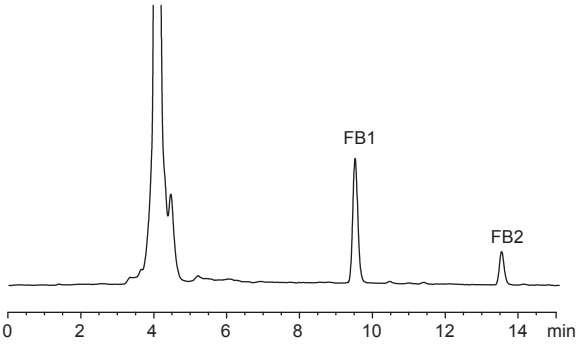

Fig 4. Chromatogram of safflower seeds sample spiked with 0.2 ug/g of FB1 and 0.07 ug/g of FB2

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