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MULTI-RESIDUE MYCOTOXIN ANALYSIS OF DRY DISTILLERS GRAINS

SINGLE RUN ANALYSIS OF AFLATOXINS, OCHRATOXIN A, ZEARALENONE AND FUMONISINS BY HPLC AND POST-COLUMN DERIVATIZATION

Distillers grains (DG) are the still residues after the ethanol has been collected. Approximately 90 % of US production is used in domestic animal feed. Any Mycotoxins present in the fresh corn can be concentrated by a factor of three. Contamination can also occur during storage. This raises concern about the potential animal and human health hazards from the use of Mycotoxin contaminated distillers grains. Corn entering the ethanol processing plant as well as distillers grains should be routinely tested for Mycotoxin contaminations to ensure compliance with guidelines set by FDA.

We present a single screen method to cover 4 families of toxins that could be present in dry distillers grains (DDG).

Sample Extraction and Clean Up

25 g of finely grounded sample is extracted with 150 mL of water/Methanol mixture (30/70). 20 mL of filtered extract is diluted with 70 mL of Phosphate Buffered Saline (PBS). Aflatoxins, Zearealenone and Ochratoxin A are isolated using AOZ Immunoaffinity column (Vicam, USA) according to the procedure from the column manufacture. Toxins are eluted with 2 x 2 mL of Methanol. Fumonisins are isolated using FumoniTest Immunoaffinity column (Vicam, USA) according to the procedure from the column manufacture. Toxins are eluted with 2 x 1.5 mL of Methanol and combined with eluant from AOZ column. Solution is evaporated to 0.5 mL and final volume is adjusted to 1 mL with Methanol.

METHOD

Analytical Conditions

Column: MYCOTOX™ reversed-phase C_{18}, 4.6x250 mm Catalog No. 1612124

Temperature: 40 °C

Flow Rate: 1 mL/min

Mobile Phase: Sodium Phosphate buffer, pH 3.5
Catalog No 1700-1108/MeOH/ACN

Post-Column Conditions

Post-Column System: Pinnacle PCX

Reactor Volume: 1.4 mL

Temperature: 60 °C

Reagent: OPA, Thiofluor, Brij 35® in GA104

Photochemical Reactor: UVE™

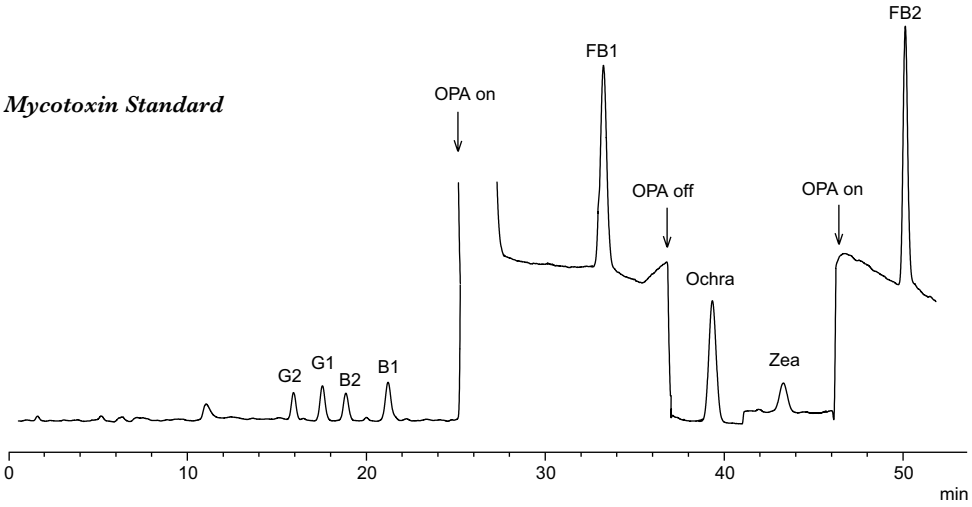
Detection:
Fluorescence
Aflatoxins (photochemical derivatization)
λ_{ex} = 365 nm; λ_{em} = 455 nm

Fumonisins (post-column derivatization with OPA)
λ_{ex} = 330 nm; λ_{em} = 465 nm

Ochratoxin A
λ_{ex} = 335 nm; λ_{em} = 455 nm

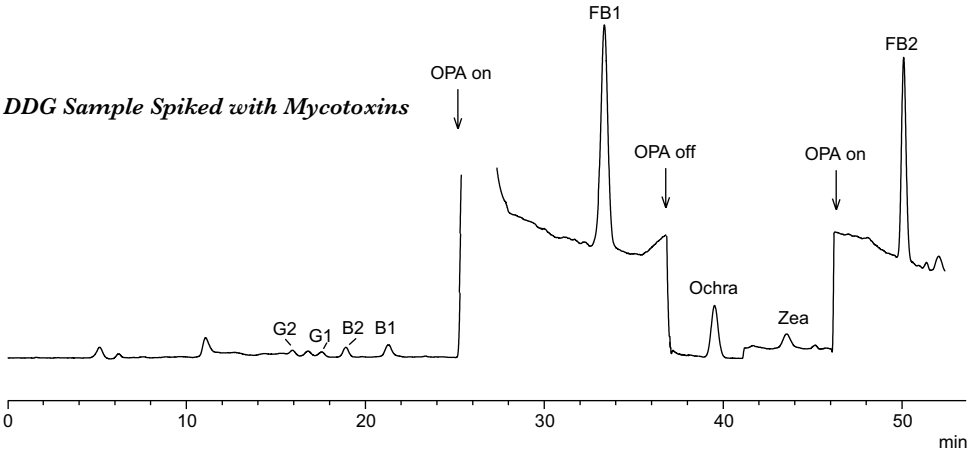
Zearalenone
λ_{ex} = 275 nm; λ_{em} = 455 nm

Mycotoxin Standard



MYCOTOXIN	SPIKE CONC, ppb	NATURAL CONTAMINATION LEVEL, ppb	RECOVERIES %	RSD N=4, %
Aflatoxin B1	10.0		65	7.6
Aflatoxin B2	3.4		79	6.3
Aflatoxin G1	10.2		75	9.4
Aflatoxin G2	4.4		82	9.1
Ochratoxin A	203		89	7.1
Zearalenone	1057	231	75	8.8
Fumonisin B1	1042	801	109	5.8
Fumonisin B2	1379	223	104	6.8

DDG Sample Spiked with Mycotoxins



5-POINT CALIBRATION CURVES		
MYCOTOXIN	CONCENTRATION RANGE	CORRELATION
Aflatoxin B1	0.23 – 113.1 ppb	0.99926
Aflatoxin B2	0.2 – 39.7 ppb	0.99966
Aflatoxin G1	0.5 – 58.2 ppb	0.99933
Aflatoxin G2	0.2 – 24.7 ppb	0.99941
Ochratoxin A	9.2 – 1155 ppb	0.99926
Zearalenone	0.024 – 12.01 ppm	0.99908
Fumonisin FB1	0.024 – 11.84 ppm	0.99987
Fumonisin FB2	0.031 – 7.84 ppm	0.99993

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