PICKERING LABORATORIES

Multi-Residue Mycotoxin Analysis—Single Run Analysis of Deoxynivalenol, Aflatoxins, Ochratoxin A, Zearalenone and Fumonisin by HPLC and Post-column Derivatization

Although Aspergillus (Aflatoxins, Ochratoxin A) are generally associated with peanuts and Fusarium (Deoxynivalenol, Zearalenone) with wheat, these fungi and those that produce other toxins are not host selective and so can cross plant species. This situation is complicated by the fact that the microscopic mold may not be visible to the naked eye. Also, when infected grains are processed, any visible mold is lost but the toxic metabolites carry over into the finished products. Thus, multi-residue analytical screens for toxins in grain and finished goods are a wiser choice than single-family protocols. We present a single screen to cover five families of toxins. This method is suitable for analyzing beverages, grains and feeds.

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.

To isolate DON 3 mL of filtered extract is mixed with 6 mL of Acetonitrile and cleaned with MycoSep 277 column (Romer Labs, USA) according to the manufacture's instructions. The cleaned solution is filtered and 5.5 mL of it is combined with eluants from AOZ and FumoniTest columns. The solution is evaporated to 0.5 mL and final volume is adjusted to 1 mL with Methanol.

Method

Analytical Conditions

Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Temperature: 40 °C

Flow Rate: 1 mL/min

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

HPLC Program			
Time	1700-1108 %	Methanol %	Acetonitrile %
0.0	85	0	15
5.0	85	0	15
5.1	57	28	15
20.0	57	28	15
23.0	40	60	0
40.0	40	60	0
50.0	20	0	80
60.0	20	0	80

Post-Column Conditions

Post-Column System: Onyx PCX and 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

UV/Vis
Deoxynivalenol
λ=218 nm

References:

Ofitserova, M., Nerkar, S., Pickering, M., Torma, L., Thiex, N., Multiresidue Mycotoxin Analysis in Corn Grain by Column High-performance Liquid Chromatography with Post-column Photochemical and Chemical Derivatization: Single-Laboratory Validation., (2009), J AOAC Int., ***92****, 15-25*

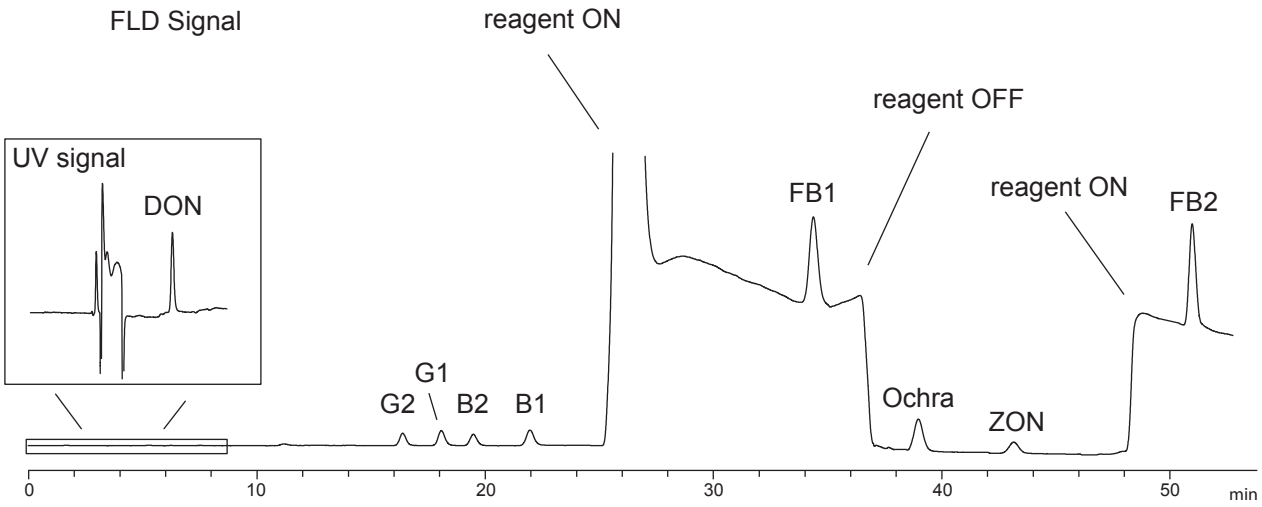

Fig 1. *Chromatogram of a standard solution of mycotoxins. Concentrations of toxins (ng/mL): deoxynivalenol (DON) - 930, aflatoxin B1 - 4.5, aflatoxin B2 - 1.6, aflatoxin G1 - 4.7, aflatoxin G2 - 2, ochratoxin A - 92, zearalenone - 481, fumonisin B1 - 474, and fumonisin B2 - 627.*

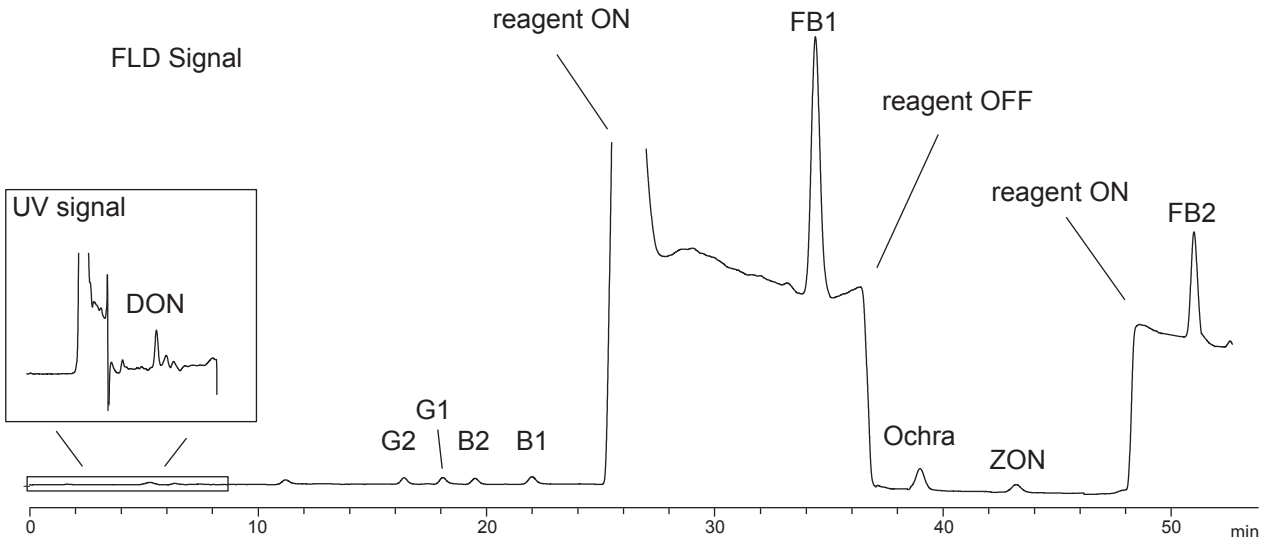

Fig 2. *Chromatogram of a corn grain sample naturally contaminated with fumonisins FB1 and FB2 and spiked with DON; aflatoxins B1, B2, G1, and G2; ochratoxin A; and zearalenone. Concentrations of toxins in the sample (ng/g): deoxynivalenol - 930, aflatoxin B1 - 5.0, aflatoxin B2 - 1.7, aflatoxin G1 - 5.1, aflatoxin G2 - 2.2, ochratoxin A - 102, zearalenone - 529, fumonisin B1 - 1838, and fumonisin B2 - 1107.*