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Multi-Residue Mycotoxin Analysis

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

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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 analysing beverages, grains and feeds.

Sample Preparation

An aliquot of the beverage or extract is evaporated to dryness to remove alcohol or any organic solvent. The residue is reconstituted in a buffer solution and (if needed) partitioned with Pentane to remove fats. Load 5 mL of the aqueous solution on a Vicam Afla/Don/Ocra/Zea/Fumo immunoaffinity column. Wash the column with 3 mL of water. Toxins are eluted with 3 mL of Methanol. The solution is evaporated to dryness and reconstituted in 0.25-2.5 mL of Methanol.

Analytical Conditions

COLUMN:	MYCOTOX™ reversed-phase C18, 4.6 x 250 mm Catalog No. 1612124
TEMPERATURE:	40° C
FLOW RATE:	1.0 mL/min
MOBILE PHASE:	• Acetonitrile • Methanol • Phosphate buffer (pH 3.3)

Post-column Conditions

POST-COLUMN SYSTEM:	Pinnacle PCX
REACTOR VOLUME:	1.4 mL
TEMPERATURE:	60° C
REAGENT:	OPA, Thiofluor in GA104
DETECTION:	• DON DAD 218 nm • Aflatoxins (Photochemical derivatization) Fluorescence λ_{ex}: 365 nm λ_{em}: 455 nm • Orchratoxin A Fluorescence λ_{ex}: 335 nm λ_{em}: 455 nm • Zearalenone Fluorescence λ_{ex}: 275 nm λ_{em}: 455 nm • Fumonisins (post-column derivatization with OPA) Fluorescence λ_{ex}: 330 nm λ_{em}: 465 nm

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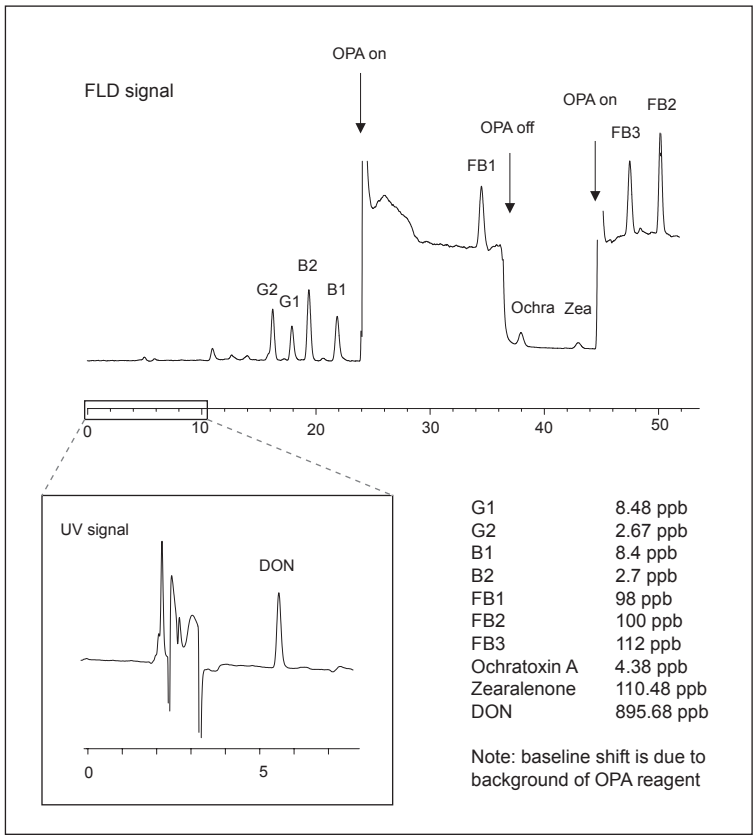
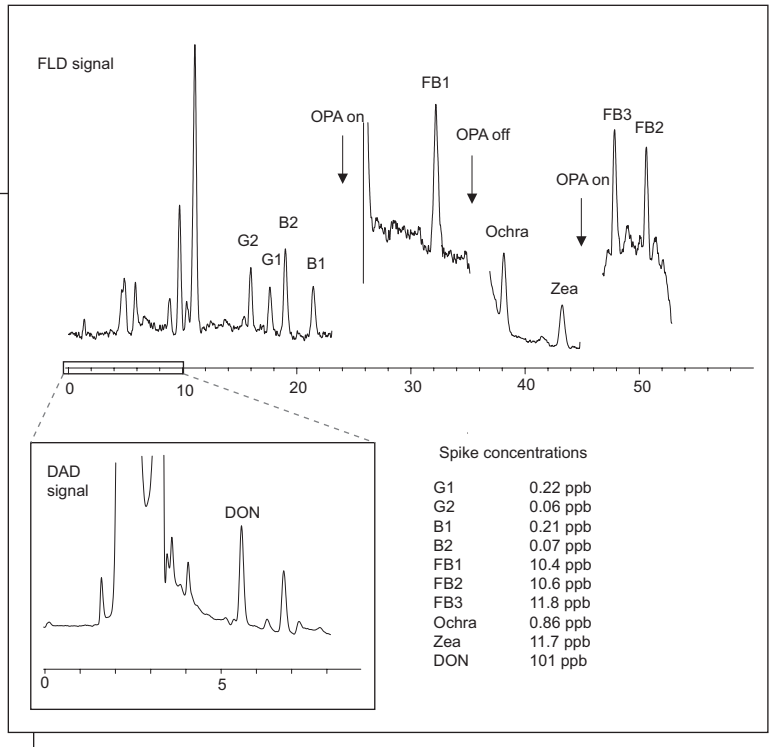
Mycotoxin Calibration Standard



G1	8.48 ppb
G2	2.67 ppb
B1	8.4 ppb
B2	2.7 ppb
FB1	98 ppb
FB2	100 ppb
FB3	112 ppb
Ochratoxin A	4.38 ppb
Zearalenone	110.48 ppb
DON	895.68 ppb

Note: baseline shift is due to background of OPA reagent

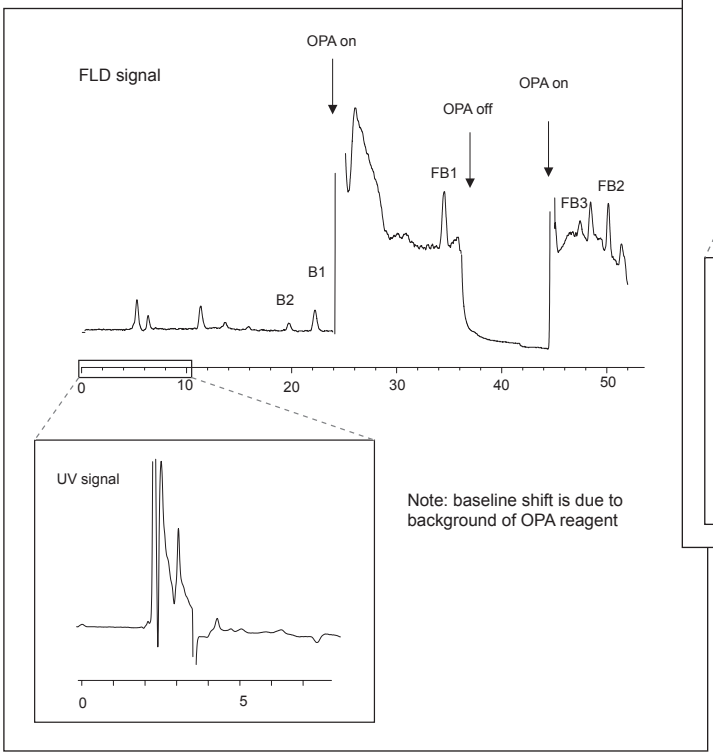
Rice Wine Sample Spiked with Mycotoxins



Spike concentrations

G1	0.22 ppb
G2	0.06 ppb
B1	0.21 ppb
B2	0.07 ppb
FB1	10.4 ppb
FB2	10.6 ppb
FB3	11.8 ppb
Ochra	0.86 ppb
Zea	11.7 ppb
DON	101 ppb

Corn Sample Naturally Contaminated with Aflatoxins and Fumonisins



Note: baseline shift is due to background of OPA reagent