PICKERING LABORATORIES

GUARANTEED CHEMISTRY

APPLICATION OF A GPC-LC-MS/MS METHOD FOR THE DETERMINATION OF 31 MYCOTOXINS IN EDIBLE OILS

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Gel Permeation Chromatography (GPC) is widely used for sample clean up in mycotoxin analysis. The most commonly described methods use GPC columns packed with SX-3 BioBeads suitable for cleaning Zearalenone, Aflatoxins and Trichothesenes from edible oils and fatty matrices. Separation of Fumonisins from the oil fraction are inadequate with this column.

The new GPC column designed by LCTech shows superior performance for simultaneous clean up of Zearalenone, Trichothesenes (types A, B and D), Aflatoxins, Ochratoxin A, including Fumonisins, along with other Mycotoxins from edible oil.

METHOD DEVELOPMENT

Different column materials and compositions have been tested for their suitability to separate the analytes from the oil fraction. Influence of the eluent composition, pH, temperature and the column capacity had been investigated. The selected material with optimized parameters was tested by a direct GPC-MS/MS coupling. The separation of the analytes from the oil was satisfactory up to oil concentration of 0.1 g/mL THF (fig1).

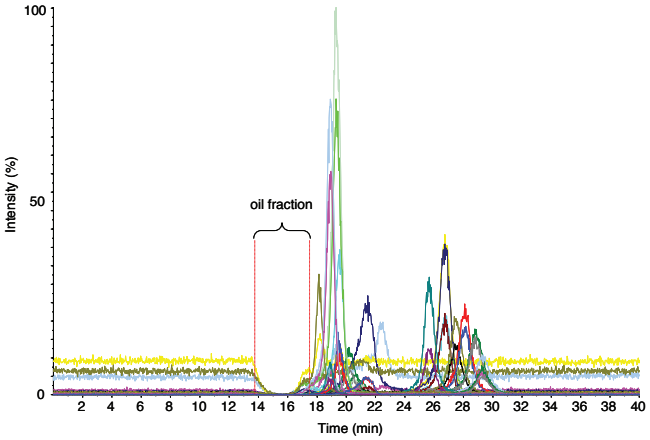

Figure 1: Chromatogram (direct GPC-MS/MS coupling) of maize oil (0.1 g/1 ml THF) spiked with a multi-mycotoxin mix

INFLUENCE OF WATER CONTENT

Variation of the water content of the eluent had a remarkable influence on the retention times of the fumonisins but had little effect on other toxins. Eluent containing 10 % of water provided good separation (fig. 2) but increasing water content to 20 % caused coelution of fumonisins with the oil fraction.

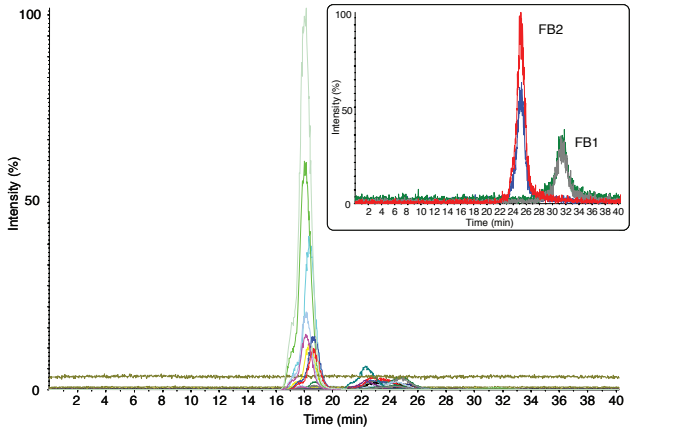

Figure 2: Multi-mycotoxin mix with eluent THF / water / formic acid 89/10/1 (v/v/v)

INFLUENCE OF PH

Variation of the pH of the eluent (0.2, 1.0 and 2.0 % formic acid) influenced the retention of fumonisins, but not of the other toxins. Best results were obtained with 1 % formic acid (fig.3). Higher acid content improved peak symmetry but FB1 and FB2 coeluted with the oil fraction.

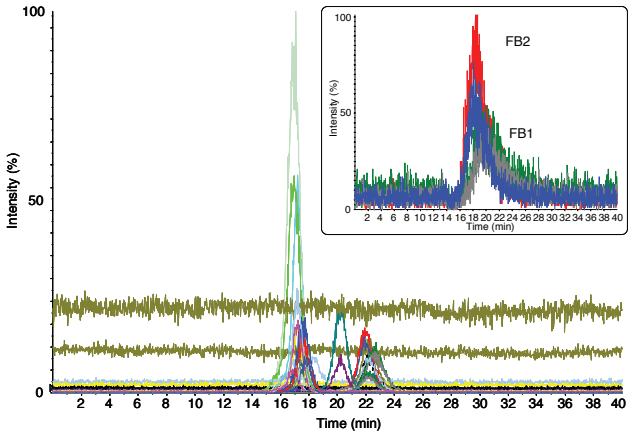

Figure 3: Multi-mycotoxin mix with eluent THF / water / formic acid 89/10/1 (v/v/v)

METHOD PARAMETERS AND PERFORMANCE

The new GPC column allows good separation of the oil and analytes (fig1). The toxins were measured by a LC-MS/MS multi-mycotoxin method with ESI+/- ionization. The recoveries obtained with the combined GPC-LC-MS/MS method ranged between 74 and 104 % with RSD less then 5 % (fig.4). For some analytes, low matrices suppression effects were observed. The limits of quantitation complied with the maximum levels for analytes regulated by the EC (1881/2006).

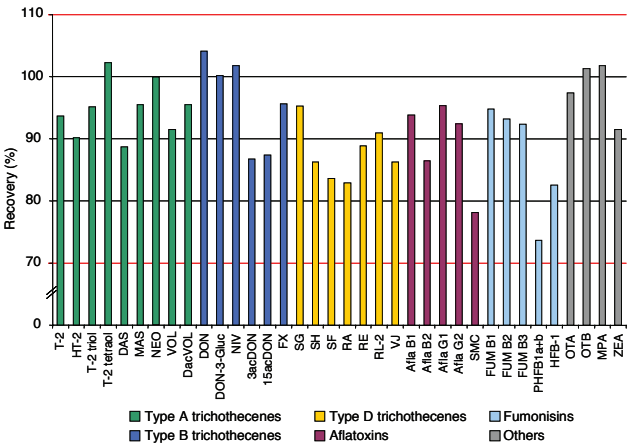

Figure 4: Recoveries from spiked corn oil for different groups of mycotoxins obtained with the final GPC-LC-MS/MS method

Final GPC-LC-MS/MS Method and Parameters

Weigh in 1 g of oil, fill up to 10 ml with THF

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Gel permeation chromatography with MykoClean™ column
- Eluent: THF/water/formic acid 89.5/10/0.5 (v/v/v)
- Flow Rate: 3 ml/min
- Injection Volume: 500 µl

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Discard fraction 0-18 min, collect 18-32 min

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Discard fraction 0-18 min, collect 18-32 min

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Adjust pH to 7 with NH_3 in methanol

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Evaporate & fill up to 1 ml with methanol/water 30/70 (v/v)

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Measure with LC-MS/MS

REFERENCES:

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