Fortis Technologies Ltd.

Application Note

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Mycotoxins

Introduction

Mycotoxins are toxic compounds that are naturally produced by certain types of moulds, these moulds grow on numerous foodstuffs such as cereals, dried fruits, nuts and spices. Mould growth can occur during harvest, during storage or on the food itself in warm, damp conditions. Most mycotoxins are chemically stable and survive food processing. Several hundred different mycotoxins have been identified, but the most common that present a hazard to human health and livestock include aflatoxins.

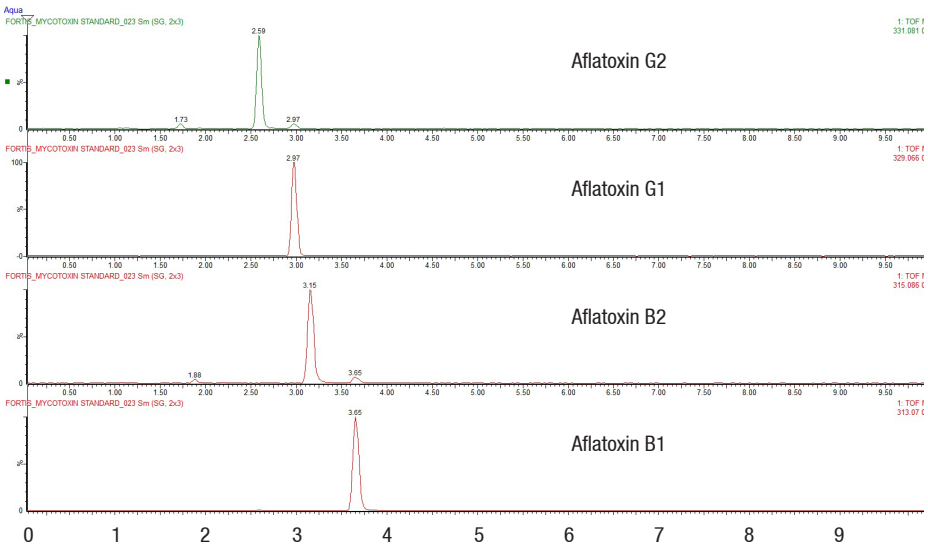
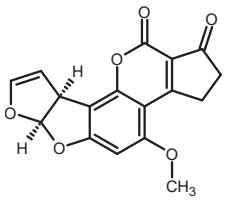


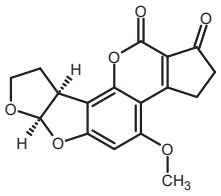
Figure 1. Separation of Aflatoxin B1, B2, G1, G2 on Evosphere AQUA

> "Mycotoxins have become a hot topic due to the adverse long term health affects, most noteably immune deficiency and cancer"

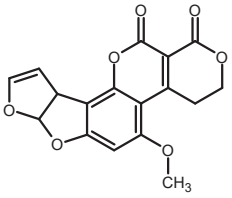
Aflatoxins are amongst the most poisonous mycotoxins and are produced by certain molds (Aspergillus flavus and Aspergillus parasiticus) which grow in soil, decaying vegetation, hay and grains. Aflatoxins have also been shown to be genotoxic, potentially damaging DNA and causing cancer in animals. There is also evidence they can cause liver cancer in humans. The maximum levels for mycotoxins in food are very low due to their severe toxicity. For example, the maximum levels for aflatoxins set by the Food and Agriculture Organisation (FAO) in various nuts, grains and dried fruits and milk are in the range of 0.5 to 15µg/Kg. Although there are 18 different aflatoxins the four most prevalent are B1, B2, G1, G2. Aflatoxin B1 is one of the most abundant and potent mutagens and carcinogens known.



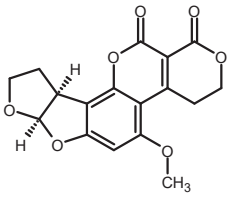
Aflatoxin B1
312.27g/mol



Aflatoxin B2
314.29g/mol



Aflatoxin G1
328.27g/mol



Aflatoxin G2
330.29g/mol

Experimental Analysis

A key criteria was to develop a simple screening HPLC method that could be used quickly and efficiently at low concentration levels for these molecules. In this application note we show the ability of the new Evosphere® AQUA column in conjunction with a simple mobile phase to produce full resolution and offer good sensitivity of isomeric molecules. We then compare this to an alternative selectivity stationary phase, Evosphere Diphenyl. On the Diphenyl column we see stronger retention for the analytes and we also note a change in elution order. This orthogonal selectivity can used to the analyst's advantage when complex mixtures are involved.

Evosphere is built around a Monodisperse Fully Porous Particle (MFPP) which is designed to provide more efficiency than traditional polydisperse particles. So in this application note a 3µm Evosphere particle is providing the efficiency and sensitivity of what would be expected if using a UHPLC sub 2µm particle, but with lower backpressure. If you run with a UHPLC particle then you get

a much elevated backpressure and the potential for blockages and robustness issues, leading to a lack of confidence in the method.

The MFPP will provide optimal packed columns, less band broadening and 40-50% greater efficiency than other equivalent silica particles in HPLC, therefore giving higher resolution and sensitivity. Bonded to this MFPP is a diverse selection of stationary phases, providing the ability to enhance resolution for critical pairs of closely related compounds, ideal for the Mycotoxins which can have very similar molecular structures.

Experimental Conditions

Columns:

3µm Evosphere AQUA 150x2.1mm

3µm Evosphere Diphenyl 150x2.1mm

Mobile phase

A: Water + 0.1% Formic acid

B: ACN + 0.1% Formic acid

Isocratic:	50:50
Flow Rate:	0.4ml/min
Temp:	35ºC
MS Detection:	Synapt G2Si QTOF
Capillary:	3kvolts
Source cone:	40volts
Desolvation temp:	350ºC
Source temp:	150ºC

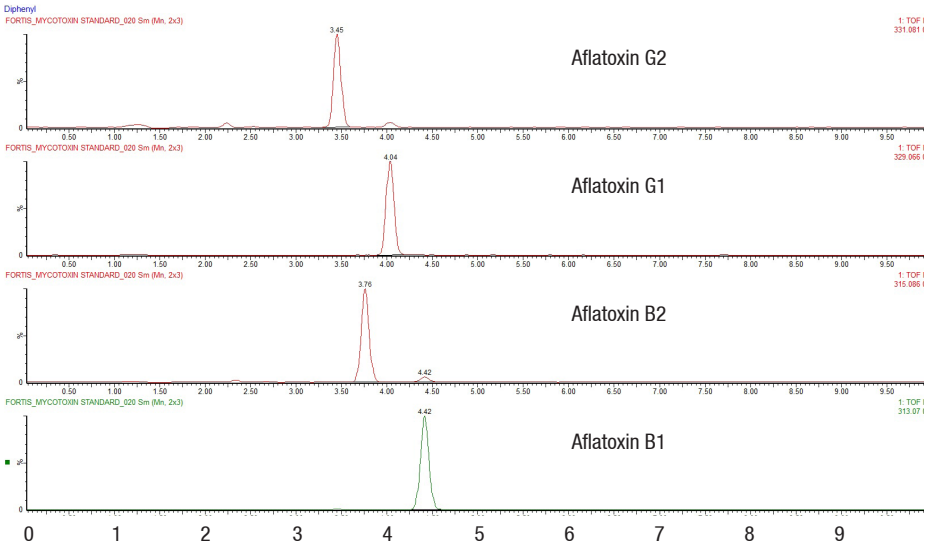


Figure 1. Separation of Aflatoxin B1, B2, G1, G2 on Evosphere Diphenyl

Conclusion

In this application note we have shown a robust LC method for separation of some of the Mycotoxins. The analysis is completed quickly in less than 5 minutes, and with good resolution between analytes, however if further gains in speed were required there is scope to decrease the size of the column or to increase the organic proportion of the mobile phase or increase the temperature to obtain a faster run time. If other metabolites were present then there is plenty of scope to alter mobile phase to gain increased resolution capacity. Sensitivity has been shown to be very good even with a 3µm particle size but could be further enhanced if required with a UHPLC particle size.

The use of a monodisperse particle has provided a significant gain in performance in terms of resolution and sensitivity for these compounds. With the simple mobile phase/ stationary phase combination used robustness will be excellent.

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