COGENT
Phenyl Hydride™
Bidentate C18™

Mycotoxins

Ochratoxin A
Zearalenone
Patulin
Aflatoxin B1, B2, G1+G
Fumonisin B1

Extended Application Note

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Introduction

The detection of mycotoxins has been an area of increasing focus in ensuring human health due to their high toxicity even at low concentrations. These chemicals are produced by various species of mold, including Aspergillus Penicillium, Paecilomyces, and Fusarium types. Exposure can come from ingestion of infested food products or, in the case of fungal species found in the home and office, inhalation.

If left unaddressed, the issue of mold-contaminated foodstuffs can produce serious effects for the consumer. Such instances have occurred in places like Kenya and India, where hundreds have died from eating infected crops. The detection of these compounds in food matrices is therefore of critical importance.

Many countries have regulations in place for detecting mycotoxins in foodstuffs, most of which call for methods based on HPLC instrumentation. The use of Cogent™ columns in these sorts of applications may be beneficial. Several types of bonded phases are available, which may be useful in obtaining unique selectivity for the different types of mycotoxins found in a sample. These include the Bidentate C18™, Phenyl Hydride™, and Diamond Hydride™. In this study, Cogent™ columns were investigated as a means to separate various mycotoxin standards. The analytes studied were ochratoxin A, zearalenone, patulin, aflatoxin B1, B2, G1 G2, and fumonisin B1.

These separations can serve as a starting point in method development for analysts working with real-world samples in the field. Although the matrices may be more complex in those instances, the same chromatographic principles will apply as when dealing with standards.

Experimental

Instrumentation

An Agilent (Little Falls, DE, USA) 1200SL Series LC system, including degasser, binary pump, temperature-controlled autosampler, and temperature-controlled column compartment was used. The mass spectrometer system was an Agilent (Santa Clara, CA, USA) Model 6210 MSD TOF with a dual sprayer electrospray source (ESI). The analytical columns were as follows:

- Cogent Bidentate C18™ 4µm 100Å, 2.1 x 100 mm
- Cogent Phenyl Hydride™ 4µm 100Å, 2.1 x 50 mm

Gradient 1:

A: DI H_2O + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid

Time (min.)	%B
0	10
10	60
12	60
14	10

Flow rate: 0.4 mL/min
Injection Volume: 1.0µL
Detection: ESI-POS MS

Samples

0.1 mg/mL stock solutions of reference standards of the analytes were prepared in a diluent of 50/50 solvent A/solvent B. The stock solutions were diluted 1:100 to obtain the working solutions. The detection of the analytes in positive ion mode is shown in the following table:

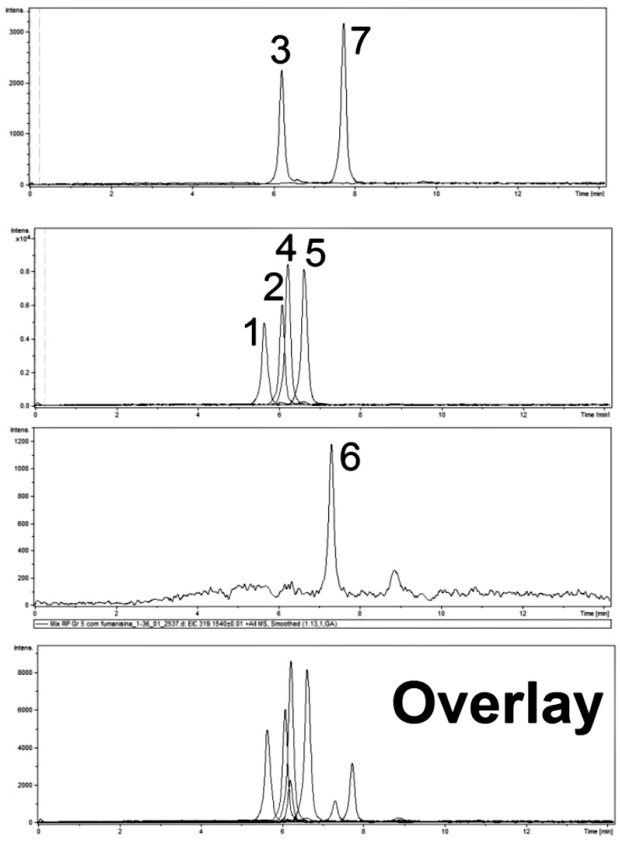
Compound name	Molecular formula	Monoisotopic neutral mass (g/mol)	$[M+H]^+$
Ochratoxin A	$C_{20}H_{18}ClNO_6$	403.81	404.0895
Zearalenone	$C_{18}H_{22}O_5$	318.36	319.1540
Aflatoxin B1	$C_{17}H_{12}O_6$	312.27	313.0707
Aflatoxin B2	$C_{17}H_{14}O_6$	314.29	315.0863
Aflatoxin G1	$C_{17}H_{12}O_7$	328.27	329.0656
Aflatoxin G2	$C_{17}H_{14}O_7$	330.29	331.0812
Fumonisin B_1	$C_{34}H_{59}NO_{15}$	721.83	722.3957

Results and Discussion

With the 2.1 x 50 mm Phenyl Hydride™ column in reversed phase, chromatographic separation could be obtained for some of the seven analytes. Additional specificity could be gained by isolating each m/z value in the EICs. Gradient 1 was used in this data. The peaks were symmetrical and showed good efficiency. The data demonstrates how Cogent™ columns can be used to separate a variety of different mycotoxins that may be found in a sample. Further method development was explored in order to try to increase the separation of the peaks.

Peaks

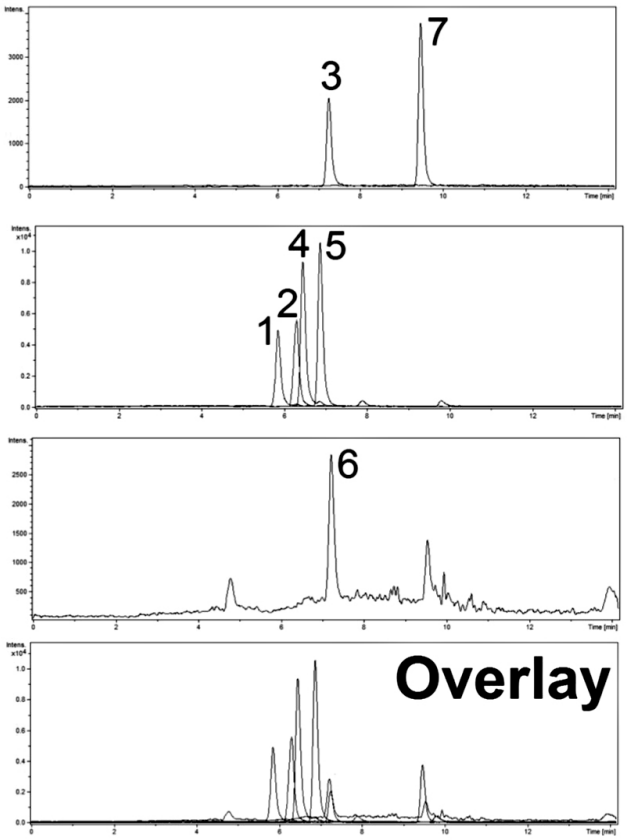
1. Aflatoxin G2
2. Aflatoxin G1
3. Fumonisin B1
4. Aflatoxin B2
5. Aflatoxin B1
6. Zearalenone
7. Ochratoxin A



Peaks

1. Aflatoxin G2
2. Aflatoxin G1
3. Fumonisin B1
4. Aflatoxin B2
5. Aflatoxin B1
6. Zearalenone
7. Ochratoxin A

Using this same gradient, a 2.1 x 100 mm Bidentate C18™ column was investigated. The data is shown below. Here, greater selectivity was observed than with the Phenyl Hydride™. The longer column length led to better resolution. Peaks were also somewhat sharper than before. The elution order was different than with the Phenyl Hydride column, allowing for alternate selectivity possibilities.



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Peaks

1. Aflatoxin G2
2. Aflatoxin G1
3. Fumonisin B1
4. Aflatoxin B2
5. Aflatoxin B1
6. Zearalenone
7. Ochratoxin A

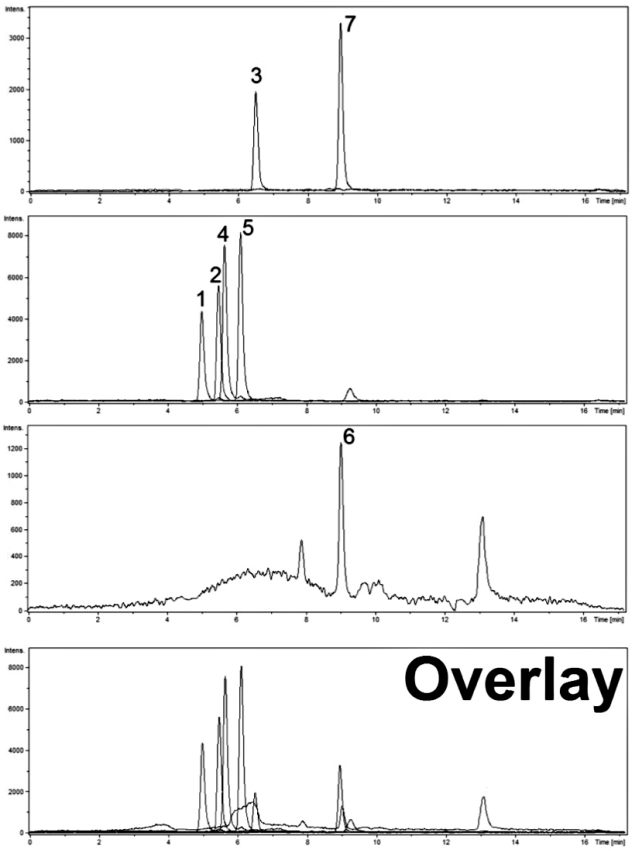
Gradient 2:

A: DI H_2O + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid

Time (min.)	%B
0	15
12	70
14	70
15	15

Flow rate: 0.4 mL/min
Injection Volume: 1.0µL
Detection: ESI-POS MS

A shallower gradient was also tried in order to obtain greater chromatographic separation. In this case, near baseline resolution was obtained for the analytes. This specificity may be important for analyses done by HPLC-UV.



Conclusion

Both Phenyl Hydride™ and Bidentate C18™ columns were investigated in order to separate mycotoxins of various types. The data shows the suitability of these columns for analyses of these compounds. Future work will encompass real-world samples that may be contaminated with molds that produce these harmful substances.

For more information visit www.mtc-usa.com

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