APPLICATION NOTE 22040

Leveraging complementary selectivity to solve challenging analytical problems

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Goal

To demonstrate the different selectivity a biphenyl chemistry column provides for the analysis of mycotoxins

Introduction

Mycotoxins, toxic metabolites produced by molds, can have serious chronic effects on the health of humans and animals. The analysis of mycotoxins of food and feed is necessary and often required by legislation. In this application, mycotoxins are used as a model suite of aromatic compounds to demonstrate why and how orthogonal chemistries can help us determine the best column choice when running a sample in a complicated matrix.

When running mycotoxin analysis on a grain or nut sample, SPE is necessary to ensure as much of the background matrix ions are removed for an accurate detection level.[1] However, there may be times when the sample preparation cannot remove all interferences from the sample, making quantitation a challenge. In these instances, a column chemistry that is orthogonal to C18 can provide confirmation for the chromatographic result.

Here we demonstrate the value of a biphenyl chemistry for the analysis of mycotoxins. Commonly, mycotoxin applications are developed around a C18 separation column, such as the Thermo Scientific™ Accucore™ C18 column. This column uses the hydrophobic properties of the target mycotoxins as the basis of separation from the matrix background. An alternate chemistry, such as the Thermo Scientific™ Accucore™ Biphenyl column will provide a different selectivity to the mycotoxins. This is because the predominant forces governing the separation in a biphenyl column are the pi-pi interactions of the electron rich stationary phase and the pi bonds of the mycotoxins.

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The structural chemistry of commonly screened mycotoxins (Figure 1) shows that there are many aromatic rings and double bonds in the mycotoxin family. From this, the Accucore Biphenyl column will provide a very different separation profile from the Accucore C18 column (Figure 2).

The change in retention may provide valuable separation between the mycotoxins present and the matrix of interest, helping deliver accurate and reproducible results.

In this application note, we showcase an example separation for a series of mycotoxins run on a Thermo Scientific™ Vanquish™ Horizon instrument coupled with a Thermo Scientific™ TSQ Quantis™ mass spectrometer and equipped with each an Accucore C18 and Accucore Biphenyl column.

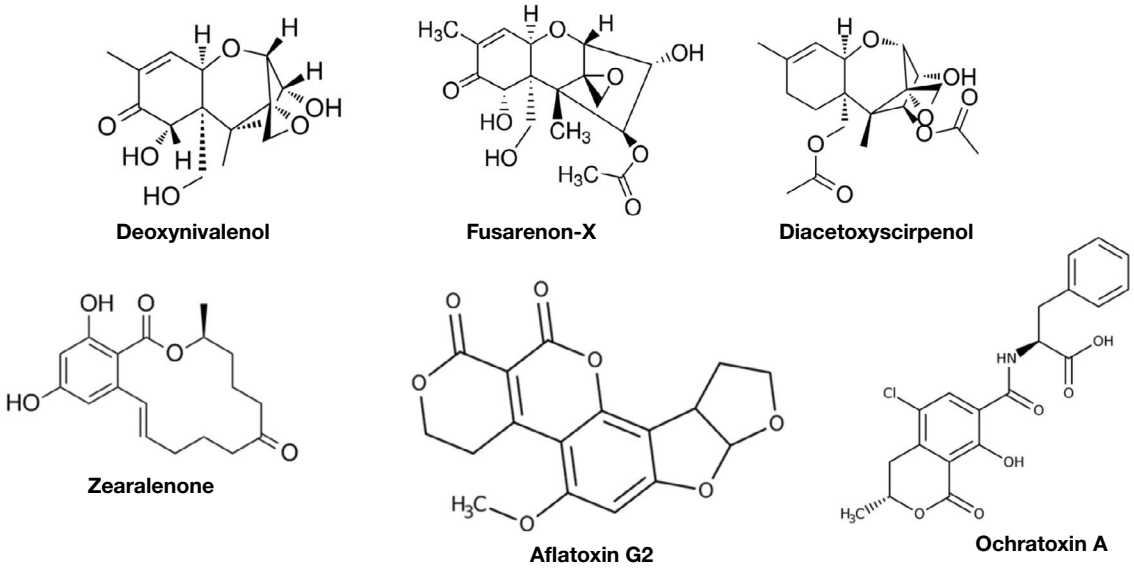

Figure 1. The structural diversity of mycotoxins makes them a model candidate to illustrate the benefit of a biphenyl chemistry for the chromatographic separation of aromatic compounds compared to a standard C18 column.

Experimental

Stock solutions of mycotoxins were diluted to target concentrations of 20 ppb in 10% methanol + 10 mM ammonium acetate, except aflatoxin G2 and B2 which were diluted to 5 ppb.

Parameter	Value
Columns	Accucore C18 2.1 x 100 mm, 2.6 µm (P/N 17126-102130) Accucore Biphenyl 2.1 x 100 mm, 2.6 µm (P/N 17826-102130)
Mobile phase A	Water with 0.5% acetic acid + 5 mM ammonium acetate
Mobile phase B	Methanol
Column temperature	40 °C
Injection volume	5 µL
Flow rate	0.4 mL/min
Gradient	See Table 1
Vials and caps	300 µL Polypropylene vial C4000-11 and PTFE/Silicone Pre-slit cap C5000-55B

Table 1. Gradient conditions used for this experiment

No	Time	Flow (mL/min)	%B	Curve
1	0.000		Run	
2	0.000	0.400	2.0	5
3	0.500	0.400	2.0	5
4	5.750	0.400	96.5	5
5	6.750	0.400	96.5	5
6	6.850	0.400	2.0	5
7	9.500	0.400	2.0	5
8	9.500		Stop run	

Parameter	Value
MS	TSQ Quantis
Ionization	HESI, positive/negative ionization modes
Mode	Timed SRM
Cycle time	0.3 s
Q1/Q3 resolution	Unit
CID gas	2.0 mTorr
SRM table	See Table 2

Table 2. SRM transitions

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)
Diacetoxyscirpenol_NH4	5.3	Positive	384.2	229.12	16
Diacetoxyscirpenol_NH4	5.3	Positive	384.2	247.13	15
Diacetoxyscirpenol_NH4	5.3	Positive	384.2	307.15	12
T-2 Toxin_NH4	5.8	Positive	484.25	185.1	22
T-2 Toxin_NH4	5.8	Positive	484.25	215.11	19
T-2 Toxin_NH4	5.8	Positive	484.25	305.14	14
HT-2 Toxin_NH4	5.4	Positive	442.2	215.11	13.5
HT-2 Toxin_NH4	5.4	Positive	442.2	263.1	12
HT-2 Toxin_NH4	5.4	Positive	442.2	323.12	9
Zearalenone_Pos	6.2	Positive	319.15	185.06	26
Zearalenone_Pos	6.2	Positive	319.15	187.08	20.5
Zearalenone_Pos	6.2	Positive	319.15	283.13	12.5
Zearalenone_Neg	6.2	Negative	317.15	131.05	29
Zearalenone_Neg	6.2	Negative	317.15	175.06	24
Zearalenone_Neg	6.2	Negative	317.15	273.11	19
Deoxynivalenol_Pos	3.1	Positive	297.13	203.11	16
Deoxynivalenol_Pos	3.1	Positive	297.13	231.1	13
Deoxynivalenol_Pos	3.1	Positive	297.13	249.11	10
Fusarenon-X_NH4	3.8	Positive	372.15	229.09	17
Fusarenon-X_NH4	3.8	Positive	372.15	247.1	14
Nivalenol	2.4	Negative	371.11	281.1	15
Nivalenol	2.4	Negative	371.11	311.1	10.5
3-Acetyl-Deoxynivalenol_Acet	4.5	Negative	397.15	59	17
3-Acetyl-Deoxynivalenol_Acet	4.5	Negative	397.15	307.12	14
3-Acetyl-Deoxynivalenol_Acet	4.5	Negative	397.15	337.13	9
Aflatoxin B1	6.8	Positive	313.07	213.05	45
Aflatoxin B1	6.8	Positive	313.07	241.05	38
Aflatoxin B1	6.8	Positive	313.07	285.07	23
Aflatoxin B2	6.6	Positive	315.09	243.06	40
Aflatoxin B2	6.6	Positive	315.09	259.06	30
Aflatoxin B2	6.6	Positive	315.09	287.09	26
Aflatoxin G1	6.5	Positive	329.07	200.05	41
Aflatoxin G1	6.5	Positive	329.07	215.07	33
Aflatoxin G1	6.5	Positive	329.07	243.07	27
Aflatoxin G2	6.25	Positive	331.08	189.05	42
Aflatoxin G2	6.25	Positive	331.08	217.08	36
Aflatoxin G2	6.25	Positive	331.08	245.08	30
Aflatoxin G2	6.25	Positive	331.08	285.07	28
Ochratoxin A	6.6	Positive	404.09	221	35
Ochratoxin A	6.6	Positive	404.09	239.01	25
Ochratoxin A	6.6	Positive	404.09	358.08	15
Fumonisin B1	4.85	Positive	722.39	334.31	39
Fumonisin B1	4.85	Positive	722.39	352.32	36
Fumonisin B2	5.3	Positive	706.4	318.32	38
Fumonisin B2	5.3	Positive	706.4	336.33	36

Results and discussion

From this workflow, the data obtained show there is a clear difference in selectivity when the C18 column is compared to the biphenyl column (Figure 2 and Table 3). The benefit of the complementary selectivity of the biphenyl column is seen in the increase of retention time. Additionally, the increase in retention time affords improved electrospray ionization efficiency of target mycotoxins.[2] From this, we can use the separation properties of the biphenyl column to further separate the mycotoxins of interest from the complicated matrix of the sample. Aflatoxins showed the greatest increase in retention times on the Accucore Biphenyl column; fumonisins show minimal change in retention times owing to these mycotoxins not having aromatic moieties in their chemical structure.

Table 3. Comparison table showing the retention time differences of an Accucore C18 column versus an Accucore Biphenyl column

Mycotoxin	RT (C18)	RT (Biphenyl)	% Change
Nivalenol	1.86	2.40	30.1
Deoxynivalenol	2.45	3.13	27.8
Fusarenon-X	3.05	3.82	25.2
3-Acetyl-Dexoynivalenol	3.60	4.48	24.4
Aflatoxin G2	3.90	6.26	60.5
Aflatoxin G1	4.05	6.49	60.2
Aflatoxin B2	4.20	6.63	57.9
Aflatoxin B1	4.34	6.85	57.8
Diacetoxyscirpenol	4.45	5.29	18.9
HT-2 Toxin	4.93	5.40	9.5
Fumonisin B1	5.02	5.16	2.8
T-2 Toxin	5.23	5.82	11.3
Ochratoxin A	5.29	6.41	21.2
Zearalenone	5.39	6.20	15.0
Fumonisin B2	5.55	5.65	1.8

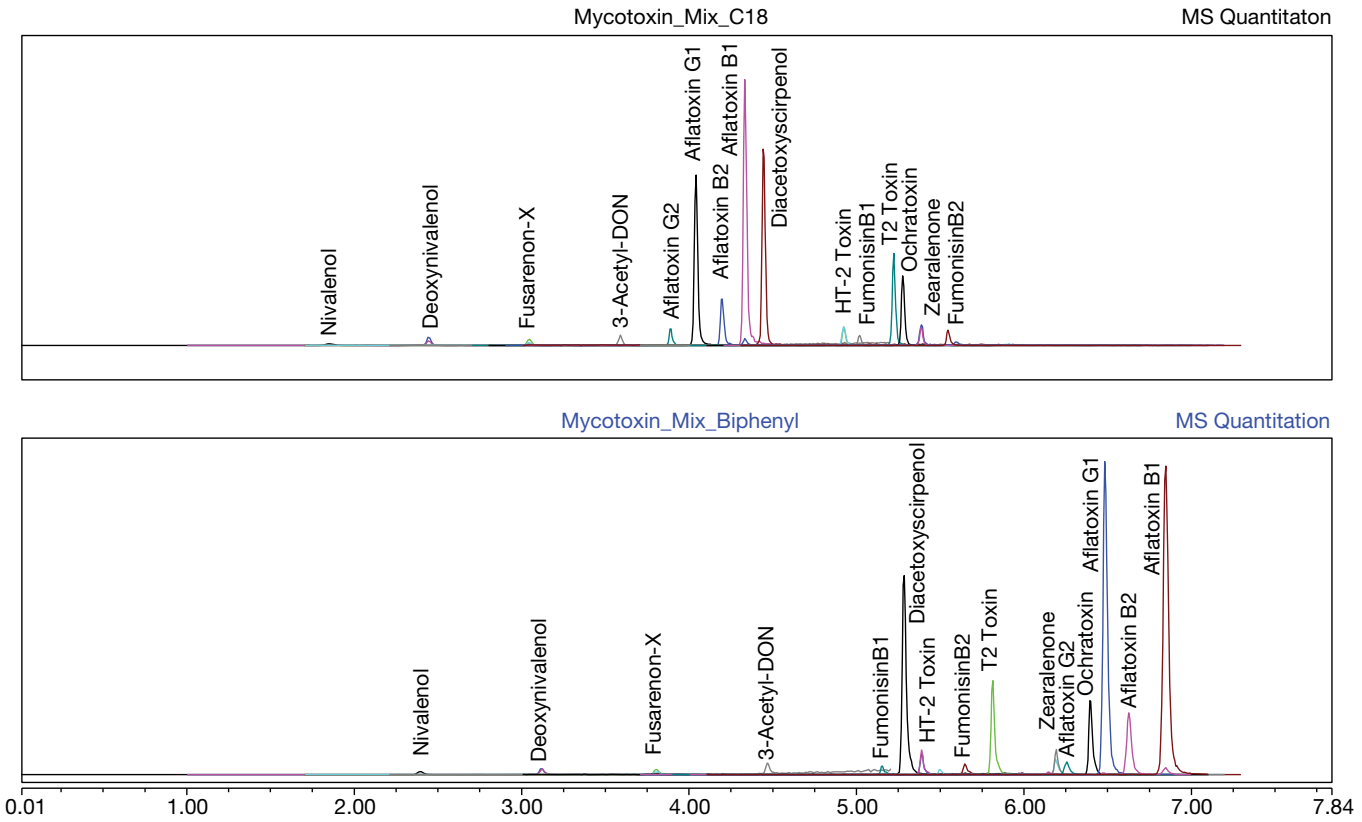

Figure 2. Comparison separation on an Accucore C18 column (top) versus an Accucore Biphenyl column (below). Additional retention afforded by the biphenyl column can be a useful tool when trying to separate compounds of interest from a food matrix.

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Conclusion

Having the ability to select from complementary chemistries will allow you to make method development choices that are more reproducible day-to-day. The Accucore column family provides a diverse selection of column chemistries to help your laboratory make the best choice for their analysis.

References

1. Thermo Fisher Scientific. Application Note 21121: Determination of Multiple Mycotoxins in Grain Using a QuEChERS Sample Preparation Approach and LC-MS/MS Detection, 2016. http://tools.thermofisher.com/content/sfs/brochures/AN-21121-SP-Mycotoxins-Grain-AN21121-EN.pdf
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