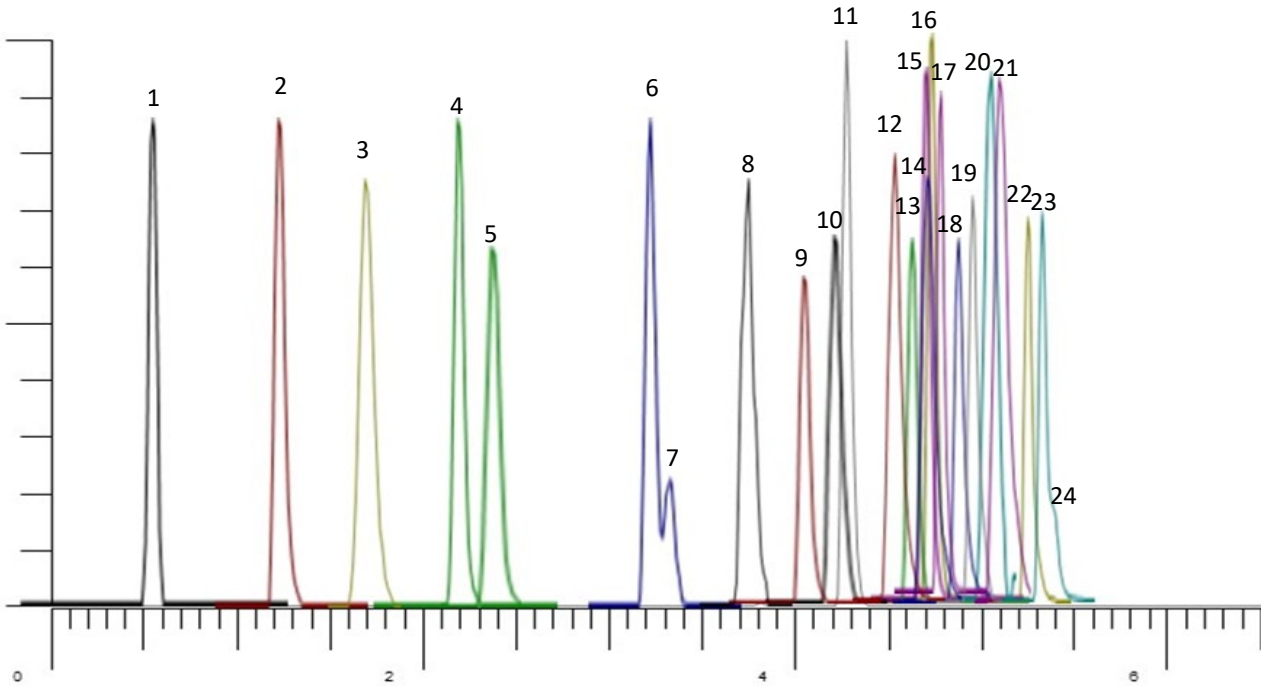
HALO® | Fused-Core® Particle Technology

Application Note: 198-M

High Throughput, High speed LC-MS/MS Separation of Mycotoxins on HALO® 2 µm PFP



TEST CONDITIONS:

Columns: HALO® 90 Å PFP, 2 µm, 2.1 x 50mm
Part Number: 91812-409
Mobile Phase A: Water/2mM ammonium formate/0.1% Formic acid
Mobile Phase B: Methanol/2mM ammonium formate/0.1% Formic acid
Gradient:

Time	%B
0.01	15
1.0	25
2.0	40
2.50	41
4.50	100
5.50	100
5.51	15
6.50	Finished

Flow Rate: 0.4 mL/min
Initial Pressure: 485 bar
Temperature: 40 °C
Injection Volume: 1 µL
Sample Solvent: 95/5 water/methanol
LC System: Shimadzu Nexera X2
Detection: +ESI MS/MS

MS CONDITIONS:

Sheath gas: 53 (arbitrary units)
Aux gas: 8 (arbitrary units)
Sweep gas: 0 (arbitrary units)
Collision energy: 35 NCE
Heater temperature: 425 °C
ESI spray voltage: 3.5 Kv.

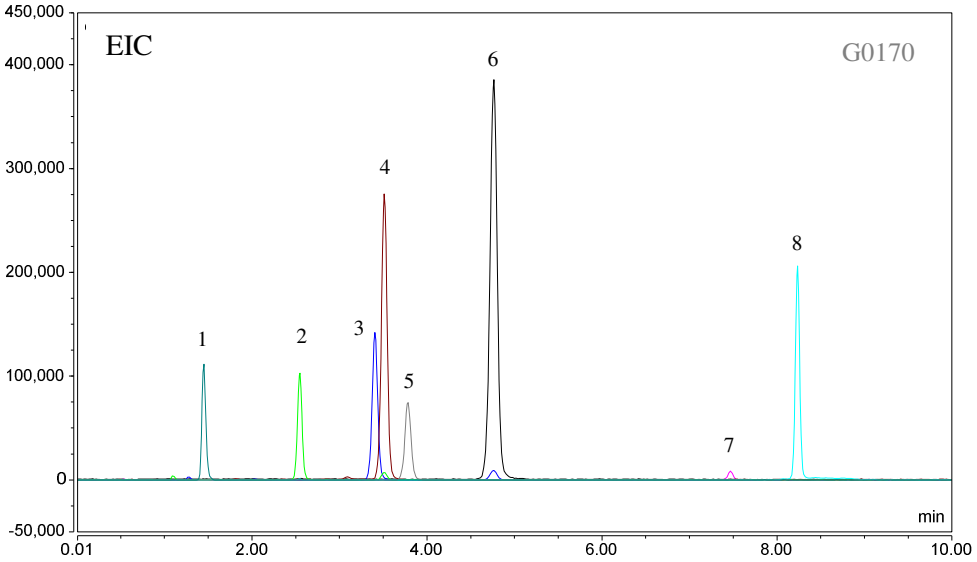
The 2 µm HALO® PFP is an ideal choice for high throughput LCMS analysis of mycotoxins, in which multiple isobaric species separation is needed. Note the separation of 24 compounds in 5.5 minutes

Peak Number	Compound	Retention Time	Precursor Ion	Product Ion
1	Nivalenol	0.71	313.1235	175.10
2	Deoxynivalenol	1.38	297.1335	249.09
3	Deoxynivalenol-3-glucoside	1.70	459.1850	193.10
4	Fusarenon X	2.37	355.1387	247.10
5	Neosolaniol	2.87	383.1702	365.16
6	15-Acetyldeoxynivalenol	3.33	339.1378	321.15
7	3-Acetyldeoxynivalenol	3.36	339.1378	231.15
8	Gliotoxin	3.97	327.0436	196.08
9	Aflatoxin G2	4.27	331.0759	312.97
10	Aflatoxin M1	4.39	329.0604	273.12
11	Aflatoxin G1	4.40	329.0601	242.90
12	Aflatoxin B2	4.44	315.0820	284.87
13	HT-2 + Na	4.47	447.1934	345.10
14	Diacetoxyscirpenol	4.49	367.2637	307.15
15	Aflatoxin B1	4.52	313.0662	286.99
16	Ochratoxin A	4.67	404.0855	238.99
17	T-2 +Na	4.72	489.2049	245.09
18	Ochratoxin B	4.88	370.1321	324.15
19	Citrinin	4.96	251.0860	233.09
20	Zearalenone	5.11	319.1491	283.08
21	Patulin +MEOH	5.11	187.0723	98.95
22	Fumonisin B1	5.24	722.3868	334.25
23	Fumonisin B3	5.41	706.3901	336.25
24	Fumonisin B2	5.44	704.3901	336.25

HALO® | Fused-Core® Particle Technology

Application Note: 176-M

LC-MS Analysis of Multiple Mycotoxins on HALO® 90 Å Biphenyl



PEAK IDENTITIES:

1. Fumonisin B1 (m/z: 722.8)
2. Aflatoxin G2 (m/z: 331.3)
3. Aflatoxin B2 (m/z: 315.3)
4. Aflatoxin G1 (m/z: 329.3)
5. Fumonisin B2 (m/z: 706.8)
6. Aflatoxin B1 (m/z: 313.3)
7. Zearalenone (m/z: 319.4)
8. Ochratoxin A (m/z: 404.8)

TEST CONDITIONS:

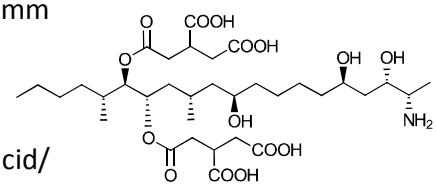
Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100mm
Part Number: 92812-611
Mobile Phase A: water with 0.1% formic acid/ 5mM ammonium formate
Mobile Phase B: acetonitrile with 0.1% formic acid/ 5mM ammonium formate

Gradient:

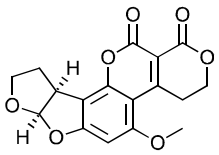
Time	%B
0.0	32
5.0	34
10.0	60

Flow Rate: 0.4 mL/min
Initial Pressure: 182 bar
Temperature: 40°C
Detection: LC-MS
Injection Volume: 2.0 µL
MS System: Thermo Fisher Orbitrap VelosPro ETD
ESI: +4
Heat Block: 350°C
Sheath Gas Flow: 34.88
Aux Gas Flow: 10.00

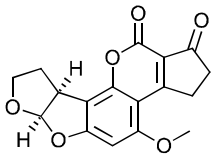
STRUCTURES:



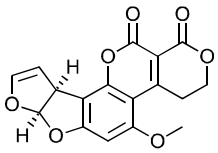
Fumonisin B1 (FB1)



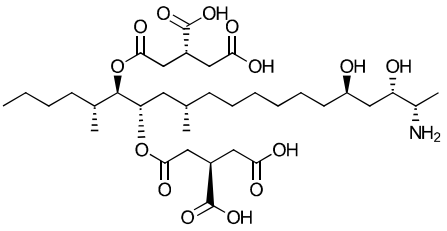
Aflatoxin G2 (AFG2)



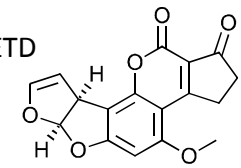
Aflatoxin B2 (AFB2)



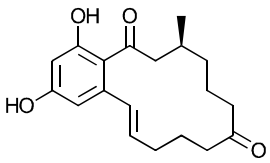
Aflatoxin G1 (AFG1)



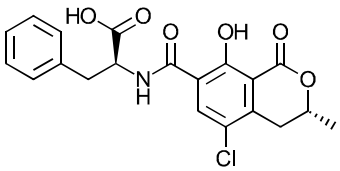
Fumonisin B2 (FB2)



Aflatoxin B1 (AFB1)



Zearalenone (ZON)



Ochratoxin A (OTA)

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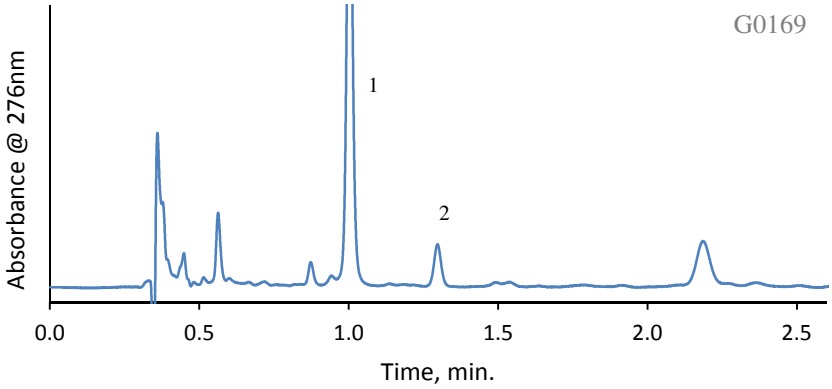
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 175-M

Separation of Patulin and HMF on HALO® 90 Å Biphenyl



PEAK IDENTITIES:

1. 5-(Hydroxymethyl) furfural
2. Patulin

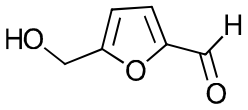
TEST CONDITIONS:

Column: HALO 90Å Biphenyl, 2.7 µm, 2.1 x 100mm
Part Number: 92812-611
Mobile Phase A: water with 0.1% acetic acid
Mobile Phase B: acetonitrile with 0.1% acetic acid
Gradient:

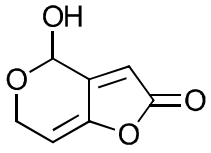
Time	%B
0.0	5
2.6	90

Flow Rate: 0.6 mL/min
Initial Pressure: 285 bar
Temperature: 40°C
Detection: UV 276 nm, PDA
Injection Volume: 1.0 µL
Sample: Apple Juice spiked with HMF and 50 ng/mL Patulin
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



5-(Hydroxymethyl) furfural



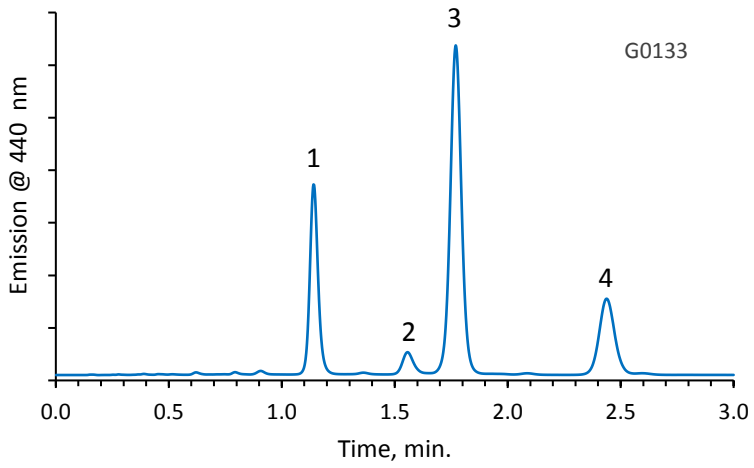
Patulin

In the United States the FDA maintains different limits for mycotoxins in many foods and beverages. Patulin, a mycotoxin that is produced from mold on a variety of fruits has a limit of 50 µg/kg. For analysis, patulin was spiked into apple juice and the sample was cleaned up using solid phase extraction. Interfering analytes such as 5-(Hydroxymethyl) furfural (HMF) can make analysis more challenging. This separation shows the two compounds separated on a HALO® Biphenyl column with enough resolution to easily check for sample recovery.

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 144-M

Isocratic Separation of Aflatoxins on HALO C18



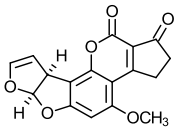
PEAK IDENTITIES:

1. Aflatoxin B1
2. Aflatoxin B2
3. Aflatoxin G1
4. Aflatoxin G2

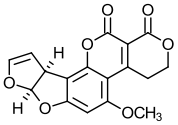
TEST CONDITIONS:

Column: HALO C18, 2.1 x 50 mm, 2.7 µm
Part Number: 92812-402
A= water
B= 50/50 acetonitrile/methanol
Isocratic: 74/26 A/B
Flow Rate: 0.8 mL/min.
Pressure: 365 bar
Temperature: 30 °C
Injection Volume: 5 µL
Sample Solvent: 70/30 water/methanol
Detection: Fluorescence Excitation - 360 nm;
Emission - 440 nm
Data Rate: 5 Hz
Response Time: 0.05 sec.
Flow Cell: 3 µL
LC System: Nexera X2

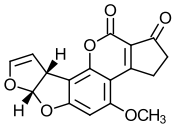
STRUCTURES:



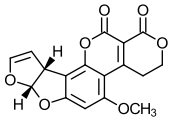
Aflatoxin B1



Aflatoxin G1



Aflatoxin B2



Aflatoxin G2

Aflatoxins are classified as mycotoxins, which are secondary metabolites produced by fungi. Under certain conditions, the fungi can grow on corn, peanuts, or tree nuts resulting in the production of aflatoxins, which are extremely toxic. A fast and sensitive method for separating four aflatoxins is demonstrated using a short HALO C18 column.

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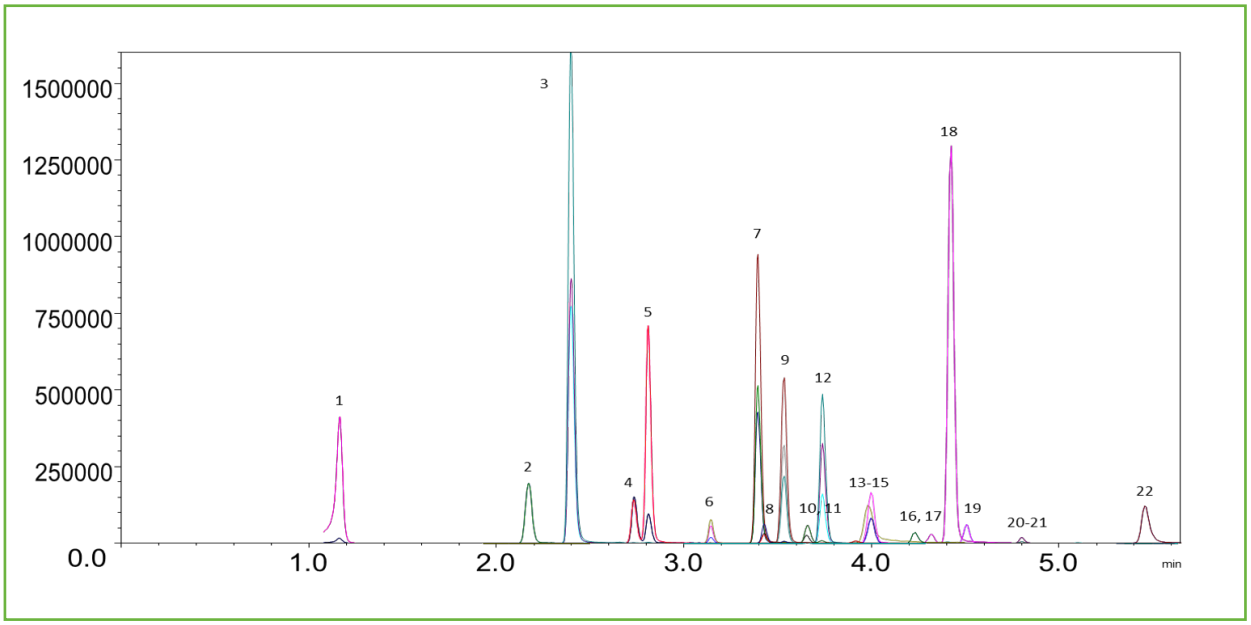
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

255-M

LC-MS Separation of Mycotoxins on HALO® PFP, 2.7 µm



Peak ID	Mycotoxin	RT (min)	Precursor	Product	Peak ID	Mycotoxin	RT (min)	Precursor	Product
1	Nivalenol	1.166	313.2	175.1	12	Aflatoxin B1	3.738	313.1	241.8
2	Fusarenone X	2.172	355.1	175.1	13	Ochratoxin B	3.916	370.1	324.1
3	Neosolaniol	2.397	399.9	185.2	14	Citrinin	3.981	251.1	233.3
4	15- acetyldeoxyniva-lenol	2.732	339.1	321.3	15	T2 Toxin	3.998	489.3	245.2
5	3- acetyldeoxyniva-lenol	2.733	339.1	231.4	16	Ochratoxin A	4.231	405.1	239.2
6	Aflatoxin M1	3.143	329.1	273.6	17	Zearalenone	4.423	319.2	283.1
7	Diacetoxyscripenol	3.394	383.9	247.5	18	Sterigmatocystin	4.506	324.3	310.2
8	Aflatoxin G2	3.427	331.1	198.1	19	Fumonisin B2	4.801	706.8	336.1
9	Aflatoxin G1	3.534	329.1	243.3	20	Fumonisin B3	4.801	706.4	336.1
10	HT2 Toxin	3.653	447.2	345.6	21	Fumonisin B1	5.102	722.4	334.2
11	Aflatoxin B2	3.661	315.1	287.2	22	Beauvericin	5.459	783.9	244.1

HALO®

ENVIRONMENTAL

TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-609
Mobile Phase A: Water, 2 mM Ammonium Formate, 0.1% Formic Acid
Mobile Phase B: Methanol, 2 mM Ammonium Formate, 0.1% Formic Acid
Gradient:

Time	%B
0.0	15
4.5	100
10.0	100

Flow Rate: 0.4 mL/min
Pressure: 280 bar
Temperature: 40 °C
Injection Volume: 7.0 µL
Sample Solvent: Methanol
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

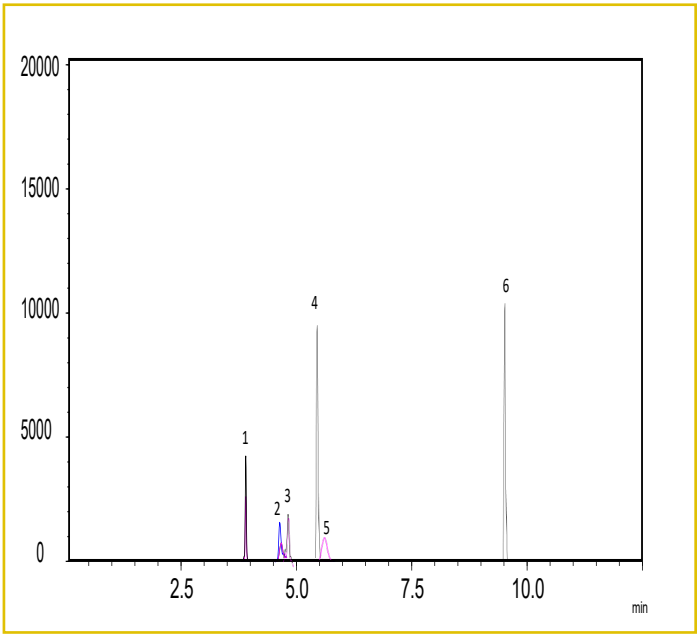
Mycotoxin contamination can have serious implications, including devastating economic losses, and human and animal death. It is imperative to successfully screen for these toxins to ensure the integrity of the food supply. Environmental analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance. The HALO 90 Å PFP can not only meet these challenges, but exceed them by demonstrating high speed and sensitivity. The HALO 90 Å PFP is an ideal column to be used in environmental, and mycotoxin analysis.

HALO®

FOOD / BEVERAGE

LCMS Screening Comparison of Mycotoxins in Craft and Home Brewed Beers

256-M

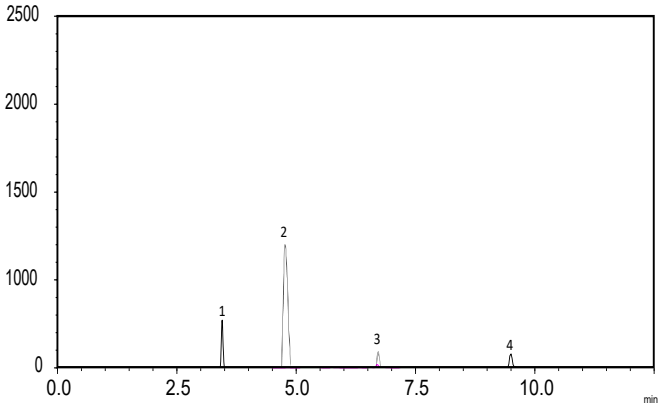


Craft Brewed Beer

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.95	489.2	245.1
2	Aflatoxin G2	4.65	331.1	189.2
3	15-acetylde-oxynivalenol	4.88	339.1	321.1
4	Aflatoxin B2	5.52	315.1	287.1
5	Aflatoxin M1	5.75	329.1	273.3
6	Zearalenone	9.55	319.1	283.2

Home Brewed Beer

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.95	489.2	245.1
2	15-acetylde-oxynivalenol	4.88	339.1	321.1
3	Aflatoxin M1	5.75	329.1	273.3
4	Zearalenone	9.55	319.1	283 .2



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PERFORMANCE YOU CAN RELY ON

HALO®

FOOD / BEVERAGE

TEST CONDITIONS:

Analytical Column: HALO 90 Å PFP, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-609
Mobile Phase A: Water, 5 mM Ammonium Formate, 0.1 % Formic Acid
Mobile Phase B: Methanol, 0.1% Formic Acid
Gradient:

TIME	%B
0	0
0.5	14
2	14
3	60
3.5	60
8	100
10	100
10.5	0
12.5	End

Flow Rate: 0.4 mL/min
Pressure: 290 bar
Temperature: 40 °C
Injection Volume: 7.0 µL
Sample Solvent: 49/50/1 ACN/H_2O/Acetic acid
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040

Mycotoxin contamination can have serious health implications. Although there are no set regulatory limits for mycotoxins in beer, most governments have clear levels for mycotoxins in various types of grain and animal feed. For example, in the United States, most levels are in the mid to high ppb range. Despite relatively low levels of mycotoxin activity in the beer, given the propensity for people to indulge in excessive drinking, and the cumulative effects of the toxicity of these compounds, excessive consumption would lead to a cumulative toxic effect, which warrants further analysis and regulation.

Beer analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance. The HALO 90 Å PFP can not only meet these challenges, but exceed them by demonstrating superior performance and sensitivity, making it an ideal column to be used in environmental, and, specifically, mycotoxin analysis.

HALO®

TECHNICAL REPORT: AMT-TR01-21-02

TITLE: **LCMS SCREENING FOR MYCOTOXINS IN BEER GRAINS AND BEER**

MARKET SEGMENT: FOOD / BEVERAGE

AUTHOR:

Andrew Harron Ph.D., Applications Scientist

ABSTRACT

Beer is one of the most widely consumed beverages on the face of the earth with millions of liters consumed every day. Beer is brewed from various types of cereal grains, which can be contaminated by mycotoxins, and as these toxins are heat stable, the possibility exists that these toxins could present in the final product, thus providing a source of mycotoxin exposure for humans. Here we present a mycotoxin screening method for beer grain samples, and both craft and home brewed beer samples, utilizing a HALO® PFP column, which enables high sensitivity, high resolution, and high-speed separations.

INTRODUCTION

Beer is one of the most consumed beverages in the world, with the average legal age person consuming approximately 27 gallons per year in the United States. The brewing of beer requires a starch source (commonly cereal grains, the most popular of which is barley), however other types of cereals (e.g., wheat, corn, rice, sorghum) can be added to the mix to cut production costs or improve the stability of the beer (1-6).

Mycotoxins, secondary metabolites that are produced by fungi, are found in various cereals. It is estimated that at least 20 % of the world's crops are infected with some kind of mold or mycotoxin (7). Thus, many grains and cereals in the brewing process can be contaminated and perpetuate this contamination into the beer itself, as most of these toxic compounds are chemically and heat stable and can survive through the entire brewing process (1,7).

Craft breweries and home brewers have skyrocketed in popularity in recent years, with over 3000 craft breweries operating in the United States alone. Craft breweries and home brewers usually incorporate a wide range of different ingredients to the brewing process, which can generate unique flavors, but can also increase the possibility of mycotoxin exposure (4-6).

Maximum contamination limits for mycotoxins in beer is still ambiguous, however, prolonged consumption of mycotoxins can have a cumulative effect on both humans and animals; therefore, even if the levels of mycotoxin exposure in beer is low, the buildup over the years can greatly enhance the toxic effects. This underscores the importance of screening for these compounds, not only in the grains, but also in the finished beer as well. Here we present a method for screening beer grain samples, and both craft and home brewed beer samples for mycotoxins, utilizing a HALO® PFP column, which enables high sensitivity, high resolution, and high-speed separations.

KEY WORDS:

Mycotoxins, LCMS, Beer, Beer grains, HALO PFP, Malt, Wheat, Brewing, Cereal

EXPERIMENTAL DATA

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Mycotoxin standards were obtained from MilliporeSigma (St. Louis, MO). Methanol (LC-MS grade), Acetonitrile (HPLC grade), acetic acid, and ammonium formate were purchased from Millipore Sigma (Burlington, MA). Nanopure water was used. Supel QuE Acetate QuEChERS salt was obtained from Supelco (Bellefonte, PA). A reversed phase superficially porous particle column from Advanced Materials Technology, Inc. (Wilmington, DE) was used: HALO 90 Å PFP, 2.7 micron (µm), 2.1 × 100 mm. The PFP stationary phase was used in this study because it has been previously shown to have superior selectivity for isomers.

Sample preparation

3 beer grain samples were obtained commercially from an online source. 1# white wheat (Home Brew Ohio, Sandusky, OH), Crystal 60L (Home Brew Stuff Inc., Boise, ID) and Gambrinus Honey Malt (LD Carlson Co., Kent, OH), were screened for mycotoxin analysis. Briefly, the grains were pulverized to a powder and then, a QuEChERS extraction was performed. After sample concentration down to 20 µL via speed vac, the sample was reconstituted in 1200 µL of 49/50/1 ACN/H_2O/Acetic acid.

Two beer samples were obtained: one was a commercial craft beer and the other was a home brewed sample, and prepped following a procedure outlined by Peters.et al. (1). Briefly, 15 mL of each sample was degassed for 30 minutes, followed by evaporation and sample concentration in a speed vac down to a volume of 20 µL. Once concentrated, the samples were subjected to QuEChERS extraction and reconstituted in 1200 µL of 49/50/1 ACN/H_2O/Acetic acid.

INSTRUMENT PARAMETERS AND GRADIENT

Analytical Column: HALO 90 Å PFP, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-609
Mobile Phase A: Water, 5 mM Ammonium Formate, 0.1 % Formic Acid
Mobile Phase B: Methanol, 0.1% Formic Acid
Flow Rate: 0.4 mL/min
Pressure: 290 bar
Temperature: 40 °C
Injection Volume: 7.0 µL
Sample Solvent: 49/50/1 ACN/H_2O/Acetic acid
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040
MS Source Conditions:
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 300 ˚C
Heat Block: 400 ˚C

Gradient			
TIME	%B	TIME	%B
0.0	0	8.0	100
0.5	14	10	100
2.0	14	10.50	0
3.0	60	12.50	End
3.5	60		

RESULTS: Grain Screening

The results of the grain screening indicated the presence of mycotoxins in all three samples of grain. Zearalenone was found in each grain sample, however at differing levels. In the white wheat sample (Figure 1), the only mycotoxin found was zearalenone (Table 1).

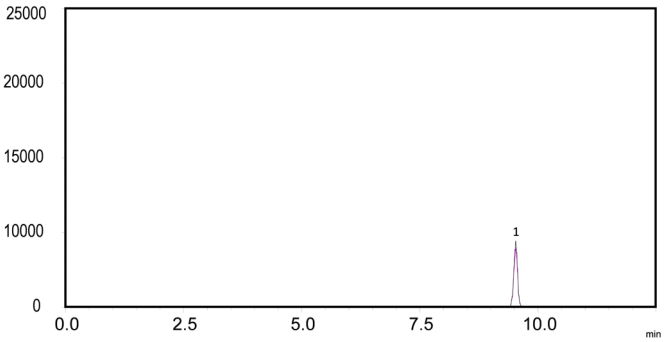
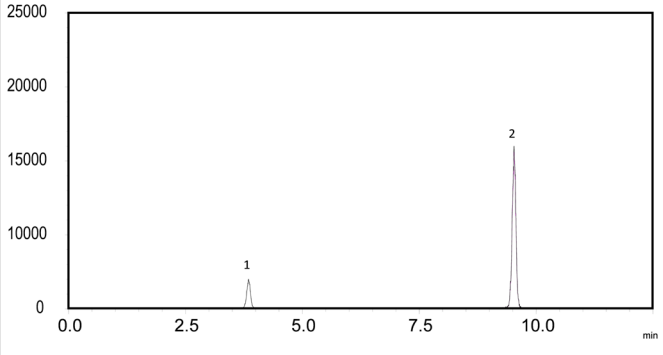


Figure 1. High resolution separation of zearalenone found in white wheat grain sample.

Table 1. Mycotoxin found in white wheat grain sample

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	Zearalenone	9.550	319.15	283.0

Figure 2 shows the mycotoxin species found in the Honey Malt grain sample which include both the T-2 toxin and zearalenone.



Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.955	489.24	245.0
2	Zearalenone	9.550	319.15	283.0

Table 2. Mycotoxins found in Honey Malt sample.

Figure 3 shows the mycotoxins found in the 60 L grain sample, containing both 15-acetyldeoxynivalenol and zearalenone.

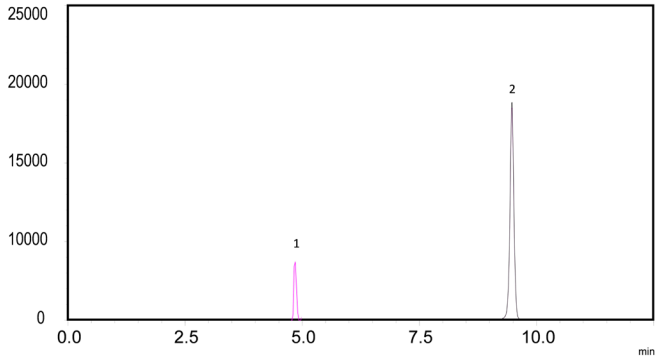


Figure 3. High resolution separation of mycotoxins found in 60 L grain sample

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	15-acetyldeoxynivalenol	4.880	339.10	321.0
2	Zearalenone	9.550	319.15	283.0

Table 3. Mycotoxins found in 60 L grain sample.

RESULTS: Beer Samples

Two beer samples were obtained and screened for mycotoxins. One sample was a commercially available craft beer, and the other sample was a home brewed sample that was donated. Figure 4, shows the results from the screening of the commercially available craft beer, which contains 6 total mycotoxins.

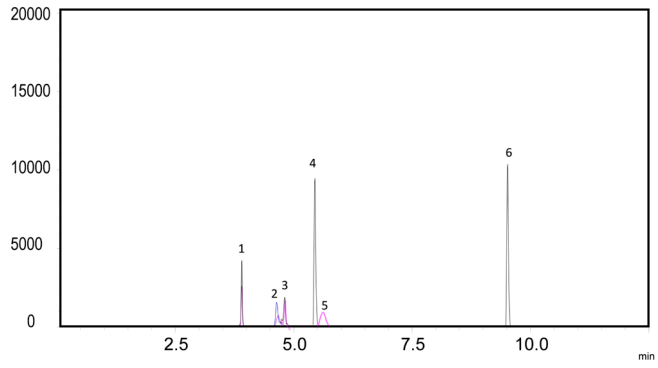


Figure 4.High resolution separation of mycotoxins found in commercial craft beer

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.955	489.24	245.0
2	Aflatoxin G2	4.650	331.10	189.0
3	15-acetyldeoxynivalenol	4.880	339.10	321.0
4	Aflatoxin B2	5.525	315.10	287.0
5	Aflatoxin M1	5.750	329.10	273.0
6	Zearalenone	9.550	319.15	283.0

Table 4. Mycotoxins found in commercial craft beer

Figure 5 shows a home brewed sample of beer which contains 4 mycotoxins.

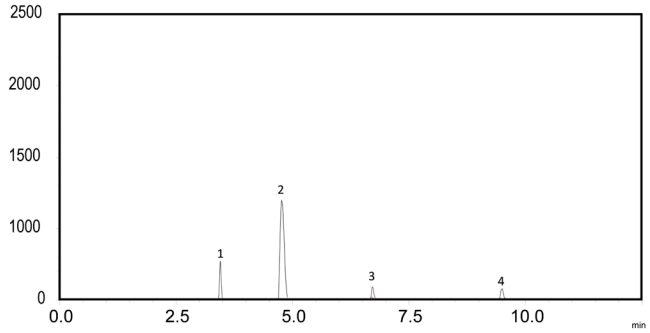


Figure 5. High resolution separation of mycotoxins found in home brewed sample

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.955	489.24	245.0
2	15-acetyldeoxynivalenol	4.880	339.10	321.0
3	Aflatoxin M1	5.750	329.10	273.0
4	Zearalenone	9.550	319.15	283.0

Table 5. Mycotoxins in home brewed sample

The commercially available craft beer contained more mycotoxins than home brewed beer, which could be expected as there are more combinations of grains and cereals that are available to a larger craft brewery than there are to home brewers. Of note, however, is that there were mycotoxins detected in both the commercial beer grains, as well as the home brew, which illustrates the importance of tighter controls on beer grains for mycotoxin testing. As these components are bought by the home brewer to formulate the beer, and mycotoxins are heat tolerant compounds, the possibility of mycotoxin exposure is high.

CONCLUSION:

Mycotoxin contamination can have serious health implications. In this technical report, 3 beer grain samples as well as two brewed samples were investigated for mycotoxin contamination, and all were found to contain mycotoxins. Although there are no set regulatory limits for mycotoxins in beer, most governments have clear levels for mycotoxins in various types of grain and animal feed. For example, in the United States, most levels are in the mid to high ppb range. Since grains and cereals are primary components of beer, it would not be unexpected to see these levels translate into beer. Despite relatively low levels of mycotoxin activity in the beer, given the propensity for people to indulge in excessive drinking, and the cumulative effects of the toxicity of these, the excessive consumption could lead to a cumulative toxic effect, which warrants further analysis and regulation.

Beer analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance. The HALO 90 Å PFP can not only meet these challenges, but exceed them by demonstrating superior performance and sensitivity, making it an ideal column to be used in environmental, and, specifically, mycotoxin analysis.

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HALO®

TECHNICAL REPORT: AMT-TR01-21-01

TITLE: **MYCOTOXIN SCREENING IN RED BELL PEPPERS**

MARKET SEGMENT: FOOD / BEVERAGE

AUTHORS:

Conner McHale, Application Technician
Andrew Harron Ph.D., Application Scientist

ABSTRACT

Mycotoxins are naturally occurring, toxic secondary metabolites produced by fungi and have been linked to numerous toxic maladies in both humans and animals. The detection of mycotoxins in the food cycle has been the cause for increasing concern, and screening for mycotoxins in food is essential to maintaining a safe food supply. Red bell peppers were screened for mycotoxins using LC-MS/MS with superficially porous particle (SPP) columns. This particle technology provides fast, high resolution separations enabling high throughput.

INTRODUCTION

Mycotoxins, produced by fungi, are an increasing problem in the food supply and can often be found in various grain species such as cereals and nuts, along with fruits and vegetables. Mycotoxins can cause serious health effects including acute poisoning, immune deficiency, and cancer. In addition, mycotoxin contamination can have a devastating economic impact to agriculture, as remediation includes crop dumping and reduced livestock production. Most of these toxic compounds are chemically stable and can survive through the entire food process, enabling a "contamination cycle" as such, initiated by contaminated grain being used as feed for livestock causing contamination to an animal, which is then positioned in the food chain. Therefore, it is critically important to screen for these compounds before the food is consumed. In this experiment we present the detection and quantification of mycotoxins found in red bell peppers from a local garden, utilizing HALO® columns, which allow high sensitivity, high resolution, and high-speed separations.

EXPERIMENTAL DATA

Mycotoxin Screening for Red Bell Peppers

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Mycotoxin standards were obtained from MilliporeSigma (St. Louis, MO). Methanol (LC-MS grade), Acetonitrile (HPLC grade), water (HPLC grade), acetic acid, and ammonium formate were purchased from Millipore Sigma (Burlington, MA). Supel QuE Acetate QuEChERS salt was obtained from

KEY WORDS:

mycotoxins, superficially porous particles, Fused-Core®, QuEChERS

Supelco (Bellefonte, PA). A reversed phase stationary phase with the following properties was tested: 2.1 × 100 mm column format, 2.7 micron (µm), 90 Å superficially porous particle packed column. The stationary phase used in this study is a pentafluorophenyl (PFP) HALO® column from Advanced Materials Technology, Inc. (Wilmington, DE).

Red bell peppers were harvested from a local garden. A modified QuEChERS procedure was performed. Briefly, 4.5 grams of homogenized food sample to 50 mL PTFE centrifuge tube, and mixed with 8 mL of ACN in 0.1% acetic acid. The contents of Supel QuE Acetate (AC) were added and vortexed for 1 minute, then spun at 3200 rpm for 5 minutes. The ACN layer was not subjected to SPE, but rather filtered through a 0.2 µm PTFE syringe filter (VWR International) and injected into the mass spectrometer.

INSTRUMENT PARAMETERS AND GRADIENT

Analytical Column: HALO 90 Å PFP, 2.7 µm, 2.1 x 100 mm

Part Number: 92812-609

Mobile Phase A: Water, 2 mM Ammonium Formate, 0.1 % Formic Acid

Mobile Phase B: Methanol, 2 mM Ammonium Formate, 0.1% Formic Acid

Gradient:

Time	%B
0.0	15
4.5	100
10.0	100

Flow Rate: 0.4 mL/min

Pressure: 280 bar

Temperature: 40 °C

Injection Volume: 7.0 µL

Sample Solvent: Methanol

Detection: +ESI MS/MS

LC System: Shimadzu Nexera X2

ESI LCMS system: Shimadzu LCMS-8040

MS Source Conditions:

Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

RESULTS:

Mycotoxin standards were used in order to optimize LCMS/MS conditions and to establish limit of detection (LOD). Furthermore, a calibration curve was performed for each standard to establish limit of quantitation (LOQ) and to quantitate any mycotoxins detected in a "real-life" sample. A total of 22 mycotoxins (**Figure 1**) are analyzed on a HALO® PFP column which is accompanied by the observed transitions (**Table 1**).

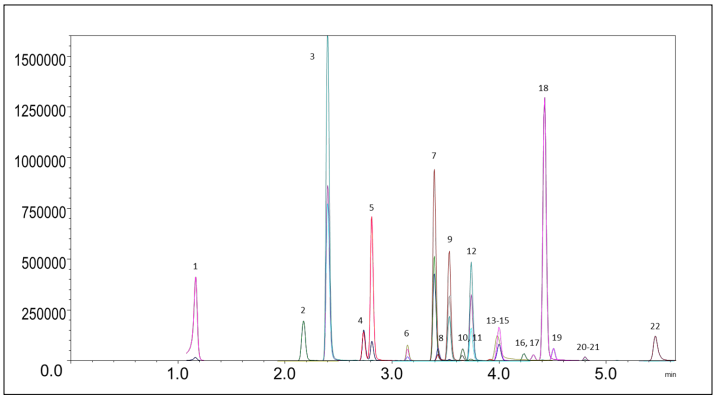


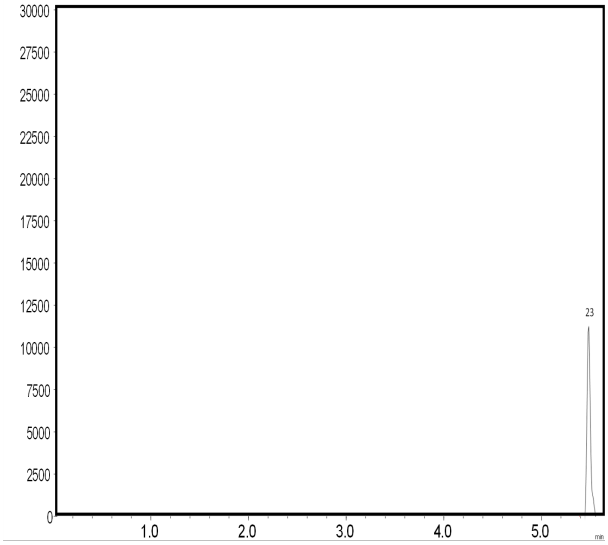
Figure 1: 22 mycotoxin standards are separated on a HALO® PFP column.

Peak Identity	*Mycotoxin*	*RT (min)*	*Precursor ion*	*Product ion*	*Collision energy (eV)*
1	Nivalenol	1.166	313	175	20
2	Fusarenone X	2.172	355.1	175	19
3	Neosolaniol	2.397	399.9	185	20
4	15- acetyldeoxynivalenol	2.732	339	321	20
5	Acetyldeoxynivalenol	2.733	339	231	20
6	Aflatoxin M1	3.143	329.1	273	23
7	Diacetoxyscirpenol	3.394	383.9	247	13
8	Aflatoxin G2	3.427	331.1	189	43
9	Aflatoxin G1	3.534	329	243	28
10	HT-2 Toxin	3.653	447.19	345	30
11	Aflatoxin B2	3.661	315.1	287	27
12	Aflatoxin B1	3.738	313.1	241	40
13	Ochratoxin B	3.916	370	324	20
14	Citrinin	3.981	251.09	233	35
15	T-2 Toxin	3.998	489.24	245	20
16	Ochratoxin A	4.231	405.1	239	20
17	Zearalenone	4.423	319.15	283	13
18	Sterigmatocystin	4.506	324.28	310	35
19	Fumonisin B2	4.801	706.83	336	35
20	Fumonisin B3	4.801	706.39	336	35
21	Fumonisin B1	5.102	722.39	334	35
22	Beauvericin	5.459	783.95	244	35

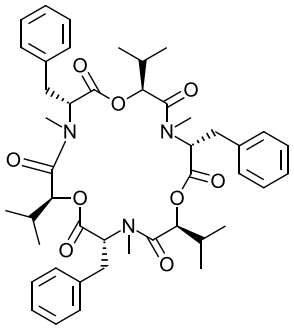
Table 1. MS/MS transitions for each analyte

Once the chromatographic method was established, optimized, and the (LOQ) was established, a modified QuEChERS procedure was performed on a red bell pepper obtained from a private garden. The only mycotoxin which was detected in quantifiable levels was beauvericin (**Figure 2**) at a concentration of 80.13 pg/mL.

Figure 2: Mycotoxin screening on red bell pepper sample. Beauvericin is detected



Beauvericin is a mycotoxin that belongs in the enniatin family, which appear in nature as mixtures of cyclic depsipeptides. The chemical structure of beauvericin can be seen in **Figure 3**.



As calibration curves were run for every standard, beauvericin was present at a concentration of 80.13 pg/mL in the red bell pepper (**Figure 4**), which although not a specified limit for beauvericin, is below limits of other regulated mycotoxins.[1]

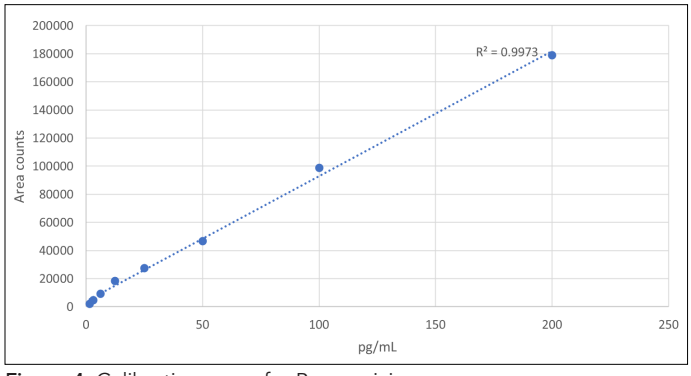


Figure 4: Calibration curve for Beauvericin

CONCLUSION:

Mycotoxin contamination can have serious implications, including devastating economic losses, and human and animal death. It is imperative to successfully screen for these toxins to ensure the integrity of the food supply. Environmental analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance.

The HALO 90 Å PFP not only meets these challenges, but exceeds them by demonstrating superior performance and sensitivity by enabling quantitation down to 80 picograms in a raw pepper sample. The HALO 90 Å PFP is an ideal column to be used in environmental and mycotoxin analysis.

REFERENCE:

1. *Istituto Superiore di Sanità (ISS), Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) and French Agency for Food, Environmental and Occupational Health & Safety (ANSES), 2018. In vivo toxicity and genotoxicity of beauvericin and enniatins. Combined approach to study in vivo toxicity and genotoxicity of mycotoxins beauvericin (BEA) and enniatin B (ENNB). EFSA supporting publication 2018: 15(5):EN-1406. 183 pp. doi: 10.2903/sp.efsa.2018.EN-1406*