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TECHNICAL REPORT: AMT-TR01-21-02

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

MARKET SEGMENT: FOOD / BEVERAGE

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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).

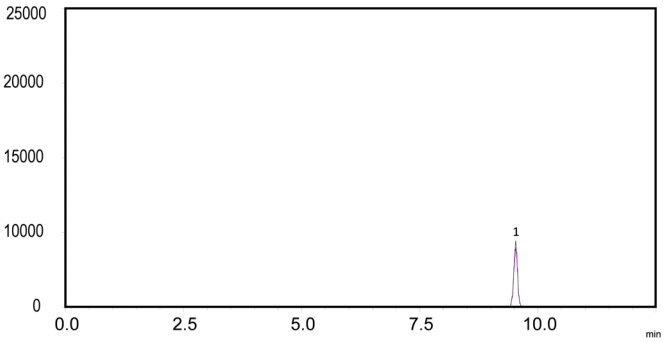
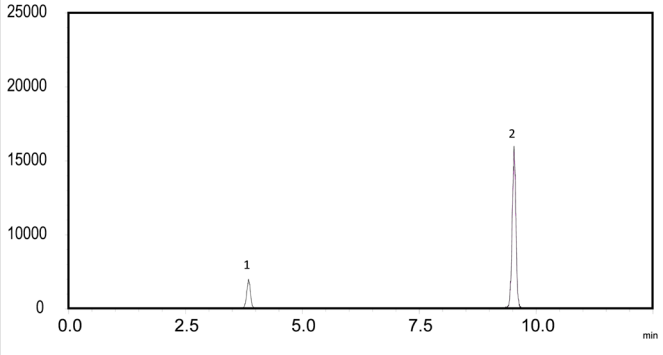


Figure 1. High resolution separation of zearalenone found in white wheat grain sample.

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.

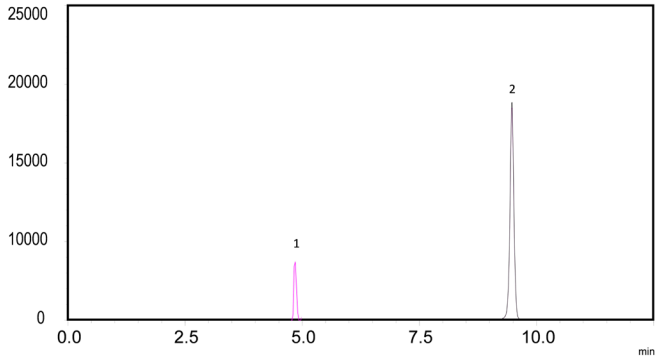


Figure 3. High resolution separation of mycotoxins found in 60 L grain sample

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.

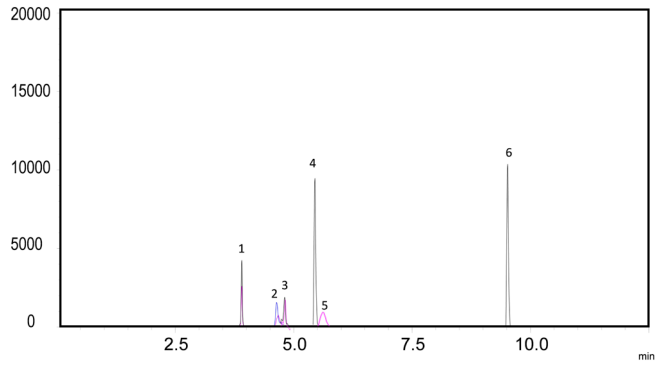


Figure 4.High resolution separation of mycotoxins found in commercial craft beer

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.

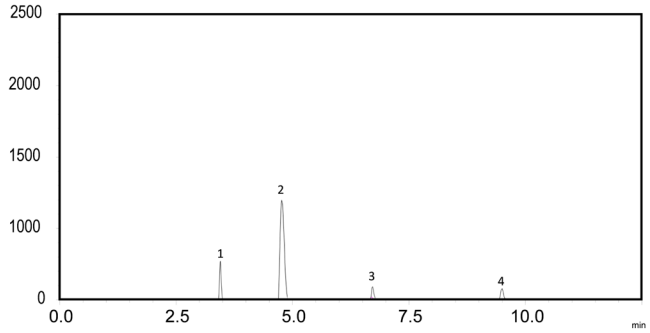


Figure 5. High resolution separation of mycotoxins found in home brewed sample

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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