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**).

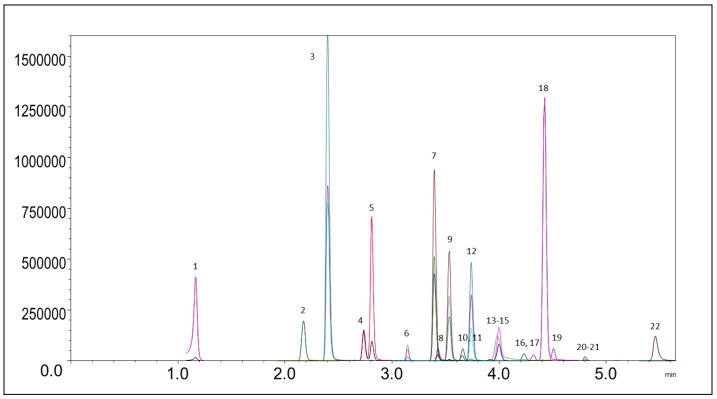


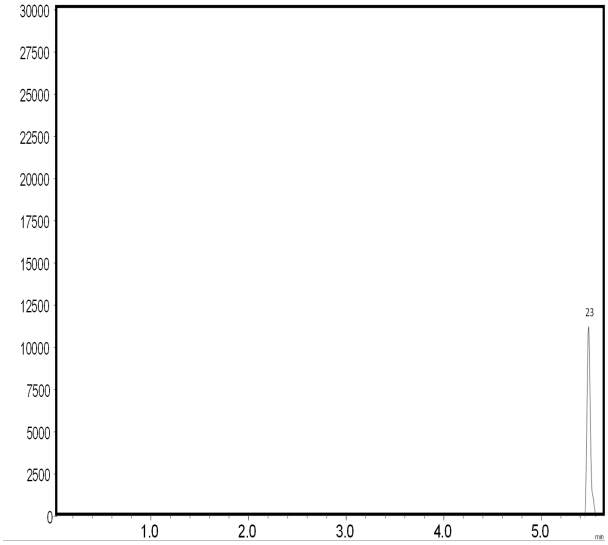
Figure 1: 22 mycotoxin standards are separated on a HALO® PFP column.

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

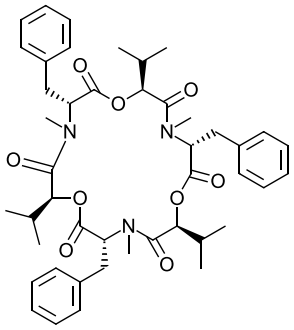
Table 1. MS/MS transitions for each analyte

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]

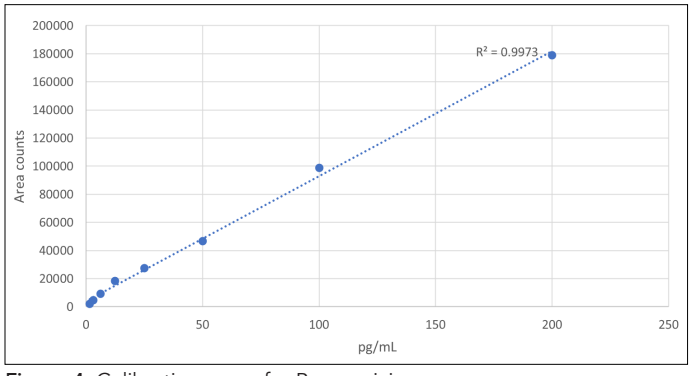


Figure 4: Calibration curve for Beauvericin

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*