Application of a Universal ELISA Method to Detect Aflatoxin B1 in Diverse Commodities with Optimized Extraction Procedures

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Introduction:

Aflatoxins are toxic fungal metabolites known to cause cancer and pose significant threats to global food security and safety. Most controlling government agencies worldwide have regulations stipulating the level of aflatoxins allowable in human and animal foodstuffs. As per the U.S. Food and Drug Administration, the allowable limit of less than 20ng/g or 20 parts per billion (ppb) is considered safe for use in all animal feeds compared to the European Union where the maximum aflatoxin B1 limit for processed food is 2 ppb. Animals are exposed to aflatoxins by consumption of feed contaminated by aflatoxin-producing fungal strains during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression, and decreased milk and egg production. Given the significant effects on human and animal health, it's critical to accurately estimate the level of aflatoxin contamination in agricultural commodities, which can exhibit high matrix effects, in order to ensure safety to the consumer.

Purpose:

To optimize extraction of aflatoxin B1 for over twenty commodities for quantification using the Helica™ Aflatoxin B1 Low Matrix ELISA from Hygiena™.

Method:

Bulk commodities, such as beans, rice, flours, corn starch, cereal, milk, dextrose, glucose, puffed food, jams, fructose syrup, maltose, oil, oatmeal, glucose, white sugar, xylitol, tapioca starch, and yogurt were obtained from the local market and were finely ground. Additionally, cannabis flower and cannabis infused samples, such as tincture, vape, and gummies were obtained from the local dispensary. Each sample was confirmed negative for aflatoxins, then fortified with aflatoxin B1 at known concentrations and extracted using different solvents for detection by competitive ELISA[1]. Optimized extraction procedures were developed and mean (%) recoveries were calculated along with (%) CV.

Results:

Ground samples were spiked at 5ng/g and/or 20ng/g followed by extraction using 70% ethanol, 70% methanol, 90% methanol, 50% acetonitrile, or 80% acetonitrile. Extraction procedures were developed and validated using the Helica™ Aflatoxin B1 Low Matrix ELISA assay. Overall % recovery was 84 - 122%. At least three independent extractions for each of the commodities were performed, which resulted in %CV of 1-16%, demonstrating the repeatability of the method.

Table 1. Spike and recovery data for commodities

Sample	Spike (ng/g)	Mean Recovery (%)	CV (%)	n	Extraction Solvent
Barley Flour	5	117	5.2	3	80% acetonitrile
Black Bean	5	111	4.9	3	80% acetonitrile
	20	92	3.6	3	
Black Rice	5	95	1.6	3	80% methanol
	20	100	3.6	3	
Cereal Milk	5	101	3.4	4	80% acetonitrile
Corn Flour	5	108	7.1	4	50% acetonitrile
	20	100	2.6	3	
Corn Starch	5	103	3.8	3	50% acetonitrile
	20	101	2.7	3	
Dextrose	5	95	6.1	3	80% acetonitrile
Fructose Syrup	5	94	5.3	4	80% methanol
Glucose	5	100	6.1	3	90% methanol
Jam	5	105	8.0	3	70% methanol
Maltose	5	110	3.0	3	80% methanol
Millet Flour	5	100	1.5	3	80% acetonitrile
Mung Bean	5	108	4.5	3	80% acetonitrile
	20	97	3.0	3	
Oatmeal	5	100	0.8	3	80% methanol
Palm Oil	5	93	4.4	3	50% acetonitrile
	20	96	4.7	3	
Puffed Food	5	106	4.0	8	80% acetonitrile
Red Rice	5	101	15.9	3	50% acetonitrile
	20	99	3.2	3	
Tapioca Starch	5	99	8.7	2	50% acetonitrile
White Sugar	5	107	11.1	3	80% methanol
Xylitol	5	122	2.2	3	70% methanol
Yogurt	5	84	2.4	3	90% methanol

Figure 1. Representative standard curve

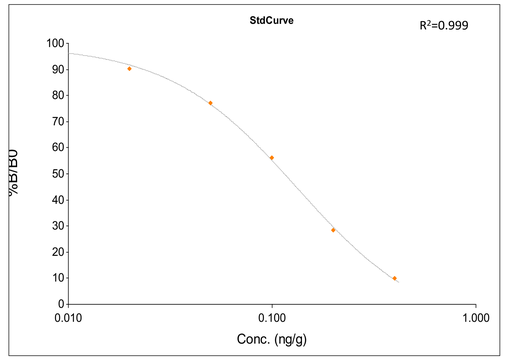

Table 2. %B/B0 from standard curve

Std (ng/ml)	%B/B0	(%) CV
0	100	3.3
0.02	90	4.7
0.05	77	0.8
0.1	56	0.6
0.2	28	1.0
0.4	10	4.0

Setting the zero standard as 100% binding (Bo), calculated % binding (%B) for each standard and sample as a percentage of the zero binding (%B/Bo)[1].

Table 3. Spike and recovery data for cannabis and cannabis infused samples

Sample	Spike (ng/g)	Mean Recovery (%)	CV (%)	n	Extraction Solvent
Cannabis Flower	20	90	11.0	9	70% ethanol
Gummy (Cannabis infused)	20	106	16.0	6	70% ethanol
Tincture Cannabis Oil	20	103	10.2	3	70% ethanol
Vape (Cannabis Infused)	20	102	3.5	3	70% methanol

Cannabis samples were obtained from local dispensary, spiked at (20ng/g) and then extracted using different extraction solvents. The method was validated by at least three independent extractions.

Significance:

The Helica™ Aflatoxin B1 Low Matrix ELISA from Hygiena™ is a universal assay fit to ensure food safety because it can accurately and reliably quantify aflatoxin B1 from such disparate sample matrices, which were successfully tested and validated using the optimized extraction procedures.

References: 1. Helica™ Aflatoxin B1 Low Matrix Package Insert. Quantitative. Version 5, March 2021.

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