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Aflatoxin Case Study

Released by:

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Job Number: XXXXX

Contains No Client Information

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Date

Client Name
Company Name
Address

Phone
Email

Dear Valued Client,

Please find enclosed the test results for your samples described as:

1. Aflatoxin Test Standards

The following tests were performed:

1) Triple Quadrupole Liquid Chromatography Mass Spectrometry (QQQ-LCMS)
2) Determination of Instrumental Detection Limit (IDL)

Objective

The goal of this project was to demonstrate the detection of a series of aflatoxins (**Figure 1**) and to determine the LOD of aflatoxins under conditions relevant to a medical device extract.

Aflatoxins are a family of mycotoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and related fungi.[1] These mycotoxins are particularly toxic toward children and can lead to stunted growth, delayed development, liver damage, and liver cancer.[2–4] There are more than 14 different known chemical species in the aflatoxin family[5] (Figure 1) which includes the four listed below as well as several metabolites. Commonalities in the family include a five-membered ring system with an aromatic core. The root cause of toxicity is believed to be intercalation into DNA as well as alkylation of base pairs leading to mutation. While no level of aflatoxin has been shown to be safe for human consumption, action levels ranging from 20-300 ppb have been established for cereal-crops intended for use as animal feed[6] and significantly lower levels for direct exposure routes.[7] Recent feedback from several regulatory agencies demonstrates increased scrutiny of medical devices for aflatoxins as well as other chemical species in the Cohort of Concern (CoC).[8]

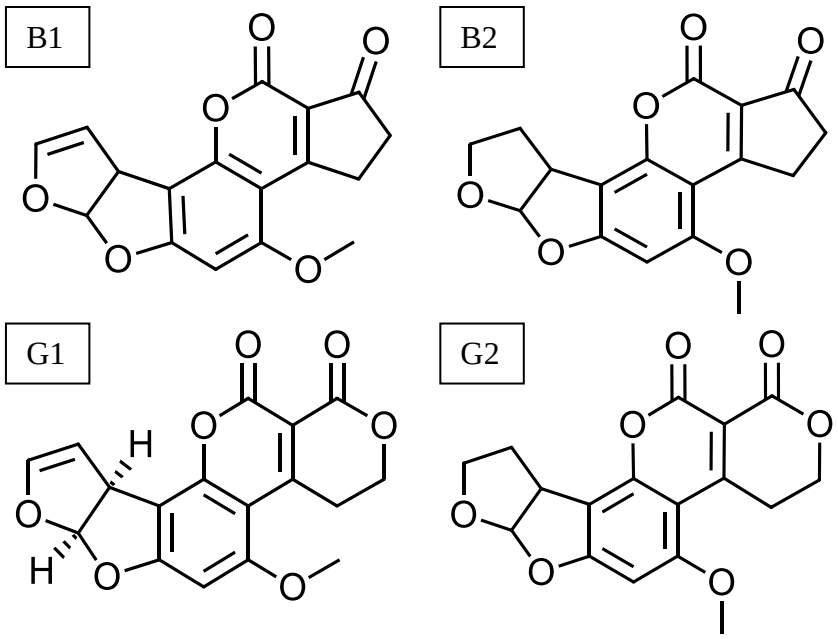

Figure 1: There are more than 14 different known chemical species in the aflatoxin family. Above are four of the most common. **Top Left:** Aflatoxin B_1**, Top Right:** Aflatoxin B_2, **Bottom Left:** Aflatoxin G_1, **Bottom Right:** Aflatoxin G_2.

(1) Cotty, P. J.; Jaime-Garcia, R. *Int. J. Food Microbiol.* **2007**, *119* (1–2), 109–115.
(2) Nogueira, L.; Foerster, C.; Groopman, J.; Egner, P.; Koshiol, J.; Ferreccio, C. *JAMA* **2015**, *313* (20), 2075.
(3) Sieber, S. M.; Correa, P.; Dalgard, D. W.; Adamson, R. H. *Cancer Res.* **1979**, *39* (11), 4545–4554.
(4) Chen, J.-G.; Egner, P. A.; Ng, D.; Jacobson, L. P.; Munoz, A.; Zhu, Y.-R.; Qian, G.-S.; Wu, F.; Yuan, J.-M.; Groopman, J. D.; Kensler, T. W. *Cancer Prev. Res.* **2013**, *6* (10), 1038–1045.
(5) Payne, G. A.; Brown, M. P. *Annu. Rev. Phytopathol.* **1998**, *36* (1), 329–362.
(6) FDA. *Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed*; Center for Food Safety and Applied Nutrition, 2000.
(7) FDA. *Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches*; 2008.
(8) EFSA. *Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree*; 2016.

Summary of Results

Four aflatoxins were analyzed using QQQ-LCMS in Multiple Reaction Monitoring (MRM) mode. Instrument detection limits ranging from 13-50 parts per trillion (ppt) were observed using this methodology. Full results for the IDL determination and spiking study are listed in **Table 1**.

Individual Test Results

A summary of the individual test results is provided below. All accompanying data, including spectra, has been included in the data section of this report.

QQQ-LCMS

IDL Determination

The IDL is the analyte concentration that is required to produce a signal greater than three times the standard deviation of the noise level within a stated confidence limit (generally 99%). This is practically measured by analyzing a standard with seven replicate injections then calculating the standard deviation from the measured concentrations of the standard. A statistical IDL determination was performed in lieu of a traditional 3:1 signal-to-noise LOD study as the instrumental noise observed by QQQ-LCMS is negligible.

To accomplish this, a stock solution of four different aflatoxins (**Figure 1**) was diluted to known concentrations ranging from 970 ppb to 30 ppt. Seven injections of a standard were performed and quantified against the calibration curves shown in **Figure 4**. Results of the IDL determination are shown in **Table 1**.

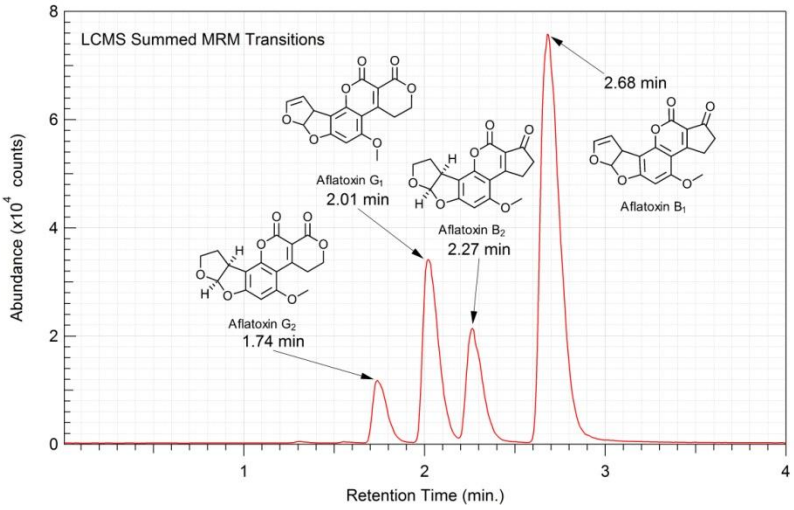

Figure 2: Overlaid chromatogram (MRM) of aflatoxin standard. RT 1.74 min: Aflatoxin G2, 94 ppb (331.2→313.1); RT 2.01 min: Aflatoxin G1, 30 ppb (329.2→311.1); RT 2.27 min: Aflatoxin B2, 30 ppb (315.0→287.1); RT 2.68 min: Aflatoxin B1, 97 ppb (313.0→285.1).

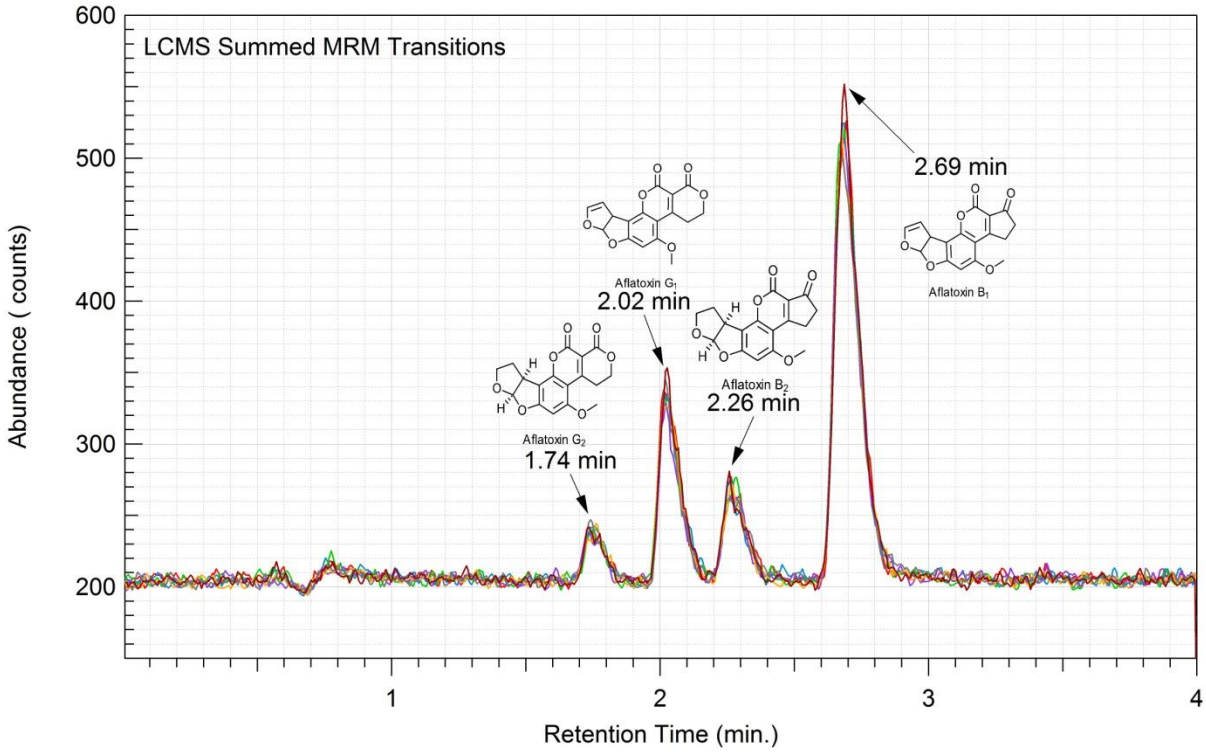

Figure 3: Overlaid chromatogram (MRM) of aflatoxin standard used for IDL determination. Red: Aflatoxin G_2, 75 ppt (331.2→313.1); Blue: Aflatoxin G_1, 235 ppt (329.2→311.1); Green: Aflatoxin B_2, 75 ppt (315.0→287.1); Magenta: Aflatoxin B_1, 243 ppt (313.0→285.1).

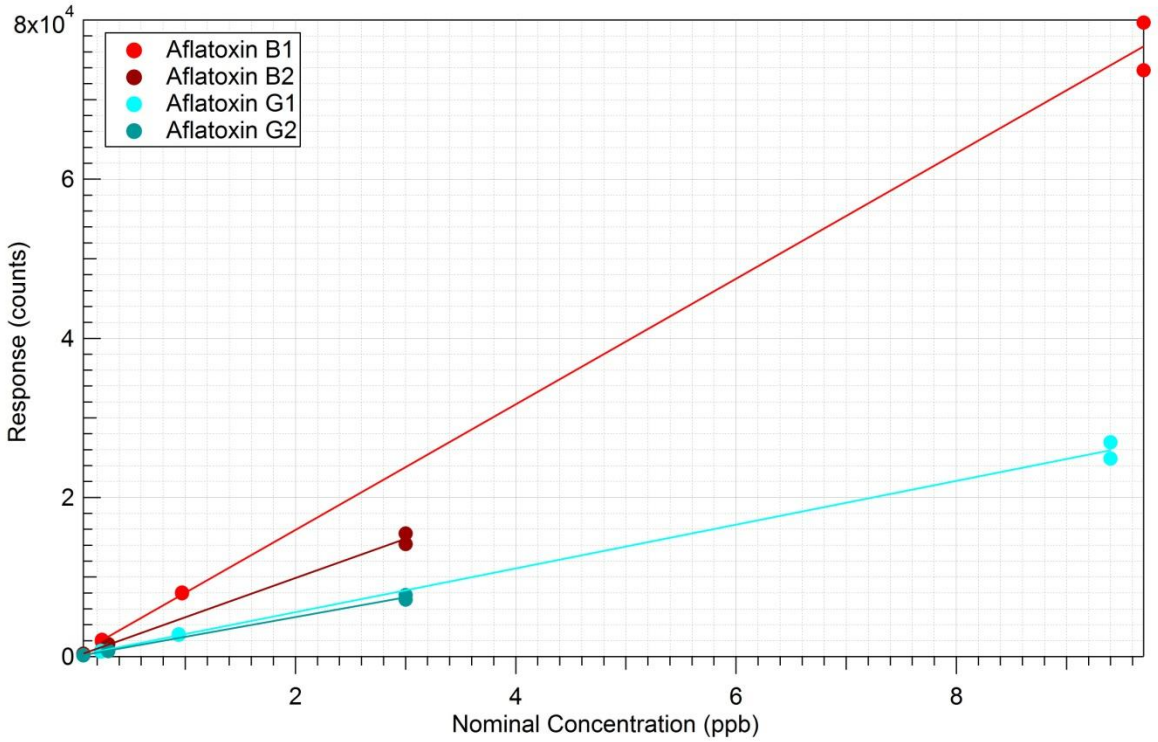

Figure 4: Aflatoxin calibration curves used for quantitation of the instrument detection limit.

Table 1

IDL Determination Results*

Aflatoxin B1			Aflatoxin B2			Aflatoxin G1			Aflatoxin G2		
Inj. #	**Peak Area**	**Conc. (ng/mL)**	**Inj. #**	**Peak Area**	**Conc. (ng/mL)**	**Inj. #**	**Peak Area**	**Conc. (ng/mL)**	**Inj. #**	**Peak Area**	**Conc. (ng/mL)**
1	2091.7	0.251	1	387.8	0.075	1	724.5	0.235	1	192.6	0.080
2	2023.1	0.243	2	328.9	0.063	2	653.0	0.209	2	176.1	0.073
3	2036.9	0.245	3	385.4	0.075	3	705.7	0.228	3	159.1	0.066
4	2010.9	0.241	4	354.5	0.069	4	619.7	0.197	4	182.2	0.076
5	1968.4	0.236	5	386.0	0.075	5	733.9	0.238	5	189.5	0.078
6	1868.5	0.223	6	367.3	0.071	6	673.1	0.216	6	184.2	0.076
7	2130.0	0.256	7	367.1	0.071	7	741.4	0.241	7	181.6	0.075
Std Dev (pg/mL)	10.592		Std Dev (pg/mL)	4.326		Std Dev (pg/mL)	16.629		Std Dev (pg/mL)	4.409	
IDL (pg/mL)	33.289		IDL (pg/mL)	13.598		IDL (pg/mL)	52.264		IDL (pg/mL)	13.858	

* All the calculated concentrations in **Table 1** are nominal concentrations unless stated otherwise.

[1]IDL = SD × 3.143 (Based on degree of freedom of 6 and confidence level of 99%)

Analysis Conditions

QQQ LCMS

This section of a Jordi report provides information on the methods used including instrument type, temperatures, solvents, sample preparation, etc. The specific conditions have been removed for this case study.

Closing Comments

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Jordi Labs specializes in polymer testing and has 30 years experience doing complete polymer deformulations. We are one of the few labs in the country specialized in this type of testing. We will work closely with you to help explain your test results and solve your problem. We appreciate your business and are looking forward to speaking with you concerning these results.

Sincerely,

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