

# Analysis of Aflatoxins Using Fluorescence Detection

Jennifer Huang and Diab Elmashni, Thermo Fisher Scientific, San Jose, CA USA

## Key Words

- Surveyor Plus™ FL Detector
- Surveyor Plus HPLC System
- TriPlus™ Autosampler
- Mycotoxins

## Introduction

Many agricultural commodities are vulnerable to attack by fungi that produce mycotoxins. These toxic substances can persist long after the fungi have been killed and contaminate foods. Most mycotoxins are stable compounds that are not destroyed during food processing or cooking. Since they can easily enter the marketplace and be a hazard to public health it is important to develop effective analytical methods for the identification and quantification of mycotoxins. Among mycotoxins, four main aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> are extremely potent carcinogens and can have significant economic impacts, making them important targets for detection and quantitation (Figure 1). In order to detect the trace amounts of these toxins that are commonly present in agricultural products it is critical to develop highly sensitive methods.

The most common methods used for aflatoxin analysis are High Performance Liquid Chromatography (HPLC) methods using fluorescence detection. This application note demonstrates the use of the Thermo Scientific Surveyor Plus HPLC System with the Surveyor FL Plus Detector, which has been optimized for trace level sample analysis. This fluorescence detector provides improved sensitivity as compared to previous technologies and enables picogram quantification of all four aflatoxins.

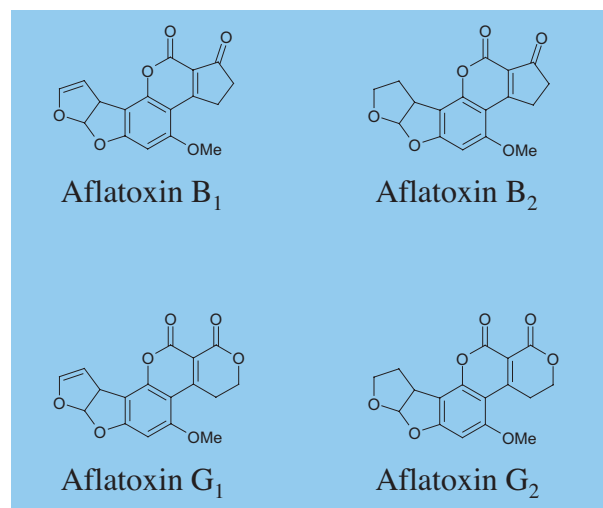


Figure 1: Chemical structures of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>

## Methods

### Sample Preparation

Aflatoxin G<sub>2</sub>, G<sub>1</sub>, B<sub>2</sub> and B<sub>1</sub> were purchased from Sigma-Aldrich (St. Louis, MO). Each of aflatoxins was diluted in methanol to 1 mg/mL solution of G<sub>2</sub>, 1 mg/mL of B<sub>2</sub>, 10 mg/mL of G<sub>1</sub>, and 10 mg/mL of B<sub>1</sub>. 100  $\mu$ L aliquot of each aflatoxin solution was then combined in a 2 mL glass vial and mixed well. This mixture was further diluted in series to 100,000 folds in water:methanol (7:3 v/v) and used as the standard solution.

### Instrumentation

TriPlus Autosampler, Surveyor™ LC Pump Plus, and Surveyor FL Plus Detector

### Chromatography Conditions

Column: Hypersil GOLD™, 3  $\mu$ m, 100  $\times$  2.1 mm  
Flow Rate: 800  $\mu$ L/min

$\lambda_{ex}$ : 365 nm

$\lambda_{em}$ : 455 nm

Mobile Phase: Water:Methanol (7:3, v/v) (isocratic elution)

Column Temperature: 40 °C

Injection Volume: 10  $\mu$ L of the prepared standard solution

Analytes: Listed in order of elution

1. aflatoxin G<sub>2</sub>, 25 pg
2. aflatoxin G<sub>1</sub>, 250 pg
3. aflatoxin B<sub>2</sub>, 25 pg
4. aflatoxin B<sub>1</sub>, 250 pg

The instruments were controlled and the data analyzed using the ChromQuest™ data system. No step changes of the excitation and emission wavelengths were used during the run.

## Results and Discussion

With improvements in column technology, aflatoxin analyses can be carried out in less than five minutes. Aflatoxins fluoresce strongly on illumination with 365 nm ultraviolet light. Figure 2 shows the fluorescence chromatogram of the four common aflatoxins with an excitation wavelength of 365 nm and an emission wavelength of 455 nm. The analytes are baseline separated with excellent resolution and quantitation at the picogram level (Table 1).

The signal-to-noise ratios (S/N) for the chromatogram in Figure 2 are calculated as the peak heights divided by the baseline noise and are listed in Table 1. Aflatoxin G<sub>2</sub> produces the strongest signal with a S/N of 1902 at a concentration of 2.5 ppb. Even though the fluorescence intensities of aflatoxins G<sub>1</sub> and B<sub>1</sub> are diminished due to the reverse-phase solvent mixture of water and methanol, their S/N ratios are still excellent and are 251 and 380 respectively.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

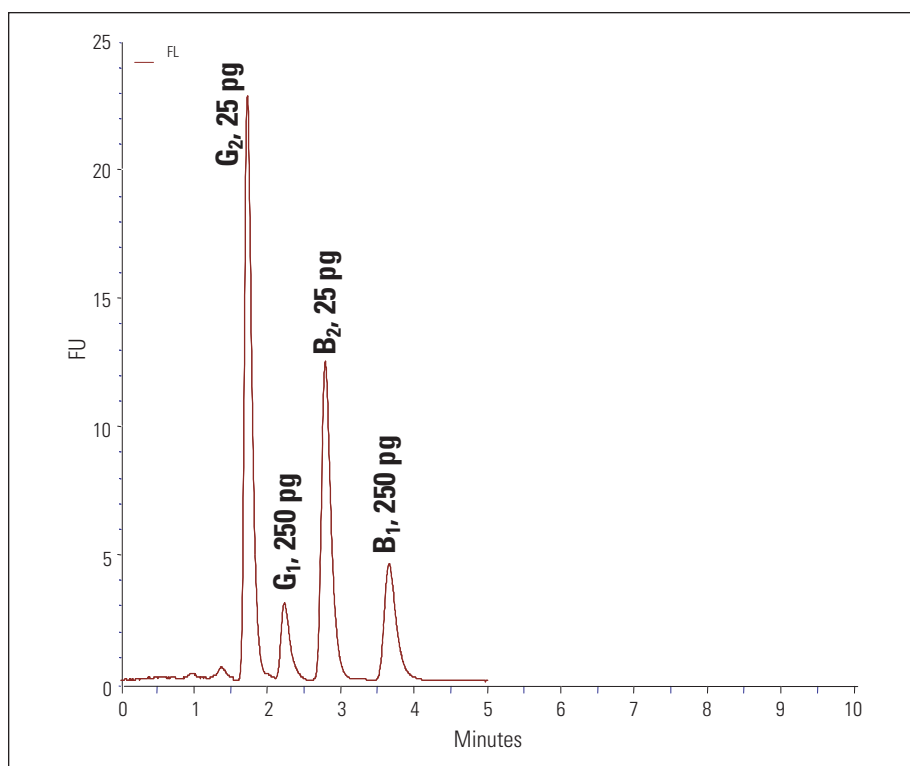


Figure 2: Fluorescence chromatogram of the four most common aflatoxins using a Thermo Scientific Surveyor FL Plus fluorescence detector with an excitation wavelength of 365 nm and an emission wavelength of 455 nm.

	<i>Aflatoxin G<sub>2</sub></i>	<i>Aflatoxin G<sub>1</sub></i>	<i>Aflatoxin B<sub>2</sub></i>	<i>Aflatoxin B<sub>1</sub></i>
Retention Time (min)	1.73	2.23	2.79	3.66
Sample Amount	25 pg	250 pg	25 pg	250 pg
S/N	1902	251	1040	380
Resolution	G <sub>2</sub> :G <sub>1</sub> = 2.51   G <sub>1</sub> :B <sub>2</sub> = 2.53   B <sub>2</sub> :B <sub>1</sub> = 3.29			

Table 1. The retention times, injected sample amounts, signal-to-noise (S/N) ratios, and resolution of the four aflatoxins obtained in Figure 2.

## Conclusion

The Surveyor FL Plus can detect aflatoxins at low ppb levels with excellent resolution and peak efficiency. The Surveyor Plus HPLC system equipped with the Surveyor FL Plus Detector enables this analysis to be performed in less than five minutes, increases separation resolution, and enables the picogram detection of all four aflatoxins tested. This instrumentation platform offers resolution and sensitivity for the quantitation of aflatoxins and is well suited for trace level analysis of fluorescent analytes.

## References

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