

Sensitive Detection of Aflatoxins by the Agilent 1290 Infinity III Fluorescence Detector



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Abstract

This application note shows the analysis of aflatoxins on the Agilent 1290 Infinity III Fluorescence Detector (FLD) at highest sensitivity, enabling low limits of detection (LODs) and limits of quantitation (LOQs) under UHPLC conditions. The 1290 Infinity III FLD offers a low-dispersion 13 µL volume cell to be used under UHPLC conditions, providing low peak dispersion volumes for optimal peak shapes. This cell enables analysis without additional derivatization. As an example, aflatoxin standards will be used to demonstrate their highly sensitive measurement, providing more efficiency and higher confidence in the obtained results.

Introduction

Aflatoxins are produced by mold fungus growing on crops under production and storage conditions. The typically naturally occurring aflatoxins are aflatoxin B1, B2, G1, and G2, and their degraded compounds aflatoxin M1 and M2. Since they are highly toxic and carcinogenic, they must be monitored due to government regulations with strict limits at the lowest possible concentration. Unfortunately, the determination of the concentration of aflatoxins by UV detection is not sensitive enough. However, aflatoxins are fluorescence active if excited at the right wavelength.

In the past, postcolumn derivatization (for example, by bromination) was required to enhance the fluorescent activity of the aflatoxins. With the highly sensitive 1290 Infinity III FLD, this step can be skipped, saving time and costs.

This application note demonstrates the measurement of aflatoxins without postcolumn derivatization at high sensitivity for low LODs and LOQs.

Experimental

Instrument setup

- Agilent 1290 Infinity III High-Speed Pump (G7120A)
- Agilent 1290 Infinity III Multisampler (G7167B)
- Agilent 1290 Infinity III Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity III FLD (G7123B) equipped with a 13 μL flow cell (p/n G7123-60500)

Software

Agilent OpenLab CDS version 2.8

Column

Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 \times 100 mm, 1.8 μm (p/n 959758-902)

Standards

- Agilent aflatoxin B1, 10 $\mu\text{g}/\text{mL}$ in acetonitrile, (p/n TOX-UNI-AFLAB1)
- Agilent aflatoxin B2, 10 $\mu\text{g}/\text{mL}$ in acetonitrile, (p/n TOX-UNI-AFLAB2)
- Agilent aflatoxin G1, 10 $\mu\text{g}/\text{mL}$ in acetonitrile, (p/n TOX-UNI-AFLAG1)
- Agilent aflatoxin G2, 10 $\mu\text{g}/\text{mL}$ in acetonitrile, (p/n TOX-UNI-AFLAG1)
- Agilent aflatoxin M1, 1 $\mu\text{g}/\text{mL}$ in acetonitrile, (p/n TOX-UNI-AFLAM1)
- Agilent aflatoxin M2, 1 $\mu\text{g}/\text{mL}$ in acetonitrile, (p/n TOX-UNI-AFLAM2)

Method

Parameter	Value
Binary Pump	
Mobile Phase A	Water
Mobile Phase B	Methanol:acetonitrile (1:1)
Stop Time	5 min
Flow Rate	0.3 mL/min
Gradient	Isocratic 40% B
Multisampler	
Injection Volume	5 μL
Needle Wash	3 s ACN
Column Oven	
Column Temperature	40 $^{\circ}\text{C}$
Fluorescence Detector	
Excitation Wavelength	365 nm
Emission Wavelength	445 nm
PMT	Standard
Peak Width	1 s response time (10 Hz)

Calibration

Stock solution: 1 $\mu\text{g}/\text{mL}$ aflatoxin G1, G2, B1, B2, M1, and M2 in acetonitrile

Dilution series: 100,000, 50,000, 20,000, 10,000, 2,000, 1,000, 200, 100, 20, 10, 2, 1, 0.2 ng/L ($\text{fg}/\mu\text{L}$). Dilution in 70:30 water/(ACN:MeOH (1:1))

Solvents

- Agilent InfinityLab acetonitrile for HPLC (p/n 5191-5100-002)
- Agilent InfinityLab methanol for HPLC (p/n 5191-5110-002)
- Agilent InfinityLab water for HPLC (p/n 5191-5120-002)

Results and discussion

The naturally occurring aflatoxins G1, G2, B1, B2, and their degradation compounds aflatoxin M1 and M2 are highly potent toxic and carcinogenic compounds. Therefore, they require ultrasensitive detection in the lower single digit ng/L (fg/ μ L) range, especially in food stuffs like peanuts or dairy products. Advantageously, they are fluorescence active compounds, which enables detection with minimized matrix

interaction at high sensitivity. To prove that the 1290 Infinity III FLD fulfills the requirements of highest sensitivity, calibration curves were measured for the mentioned aflatoxins over five decades starting at 100,000 down to 1 ng/L (fg/ μ L). Figure 1A displays an overlay of 1, 2, 10, and 20 ng/L and Figure 1B an overlay of 100,000, 50,000, 20,000, and 10,000 ng/L for all six aflatoxins measured by FLD. The measurement was done under UHPLC conditions with a 2.1 mm i.d. column and sub-2 micron material within a total run time of 5 minutes.

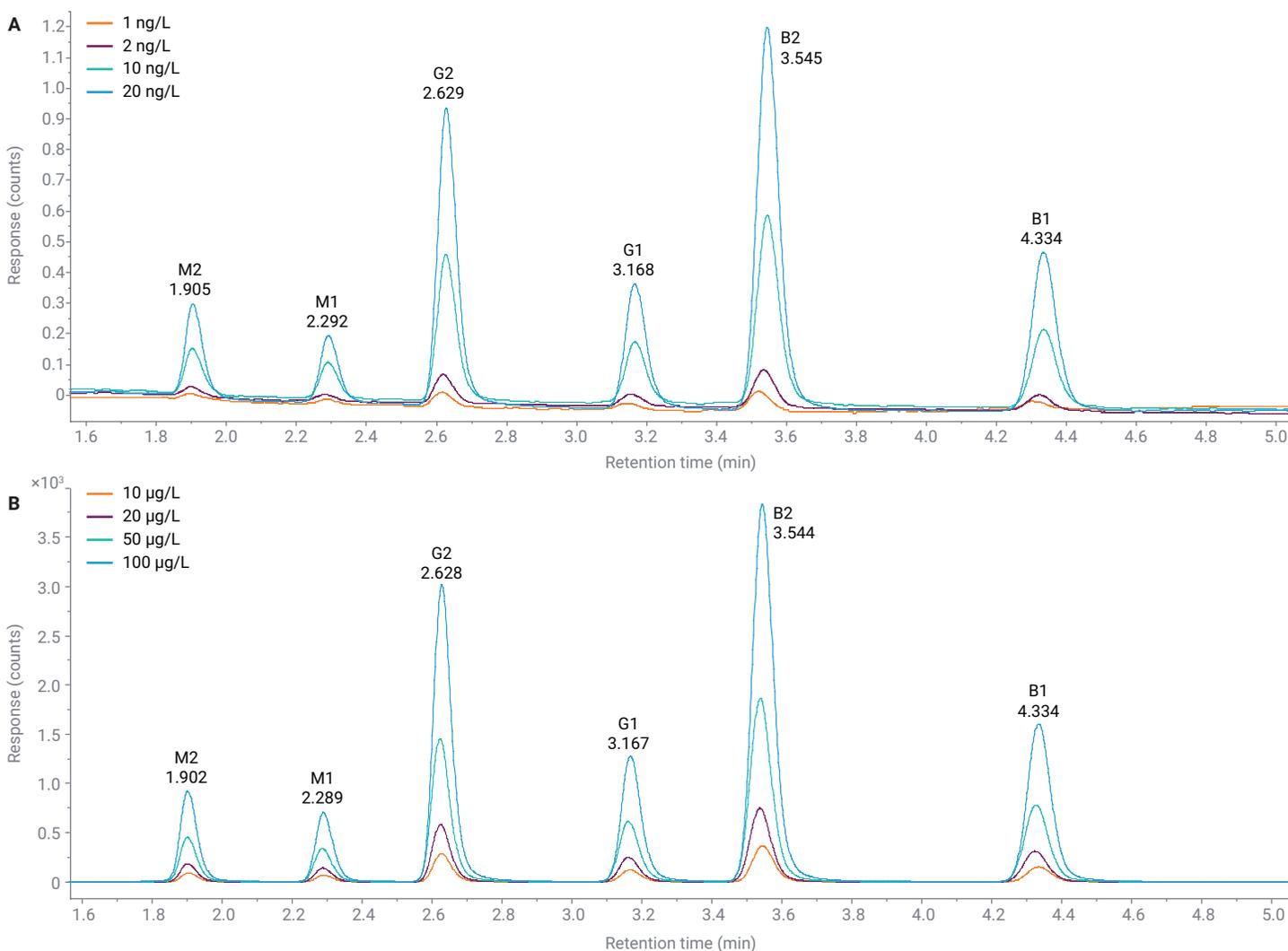


Figure 1. Overlay of the fluorescence detection measurement of aflatoxin G2, G1, B2, B1, M1, and M2 for the concentrations of: (A) 1, 2, 10, and 20 ng/L, and (B) 10, 20, 50, and 100 μ g/L.

The respective calibration curves for the aflatoxins were measured in the range from 1 to 100,000 ng/L (Figure 2). All R² values were at 0.99998 or higher.

For the calculation of the LOD and LOQ, the 1 and 2 ng/L calibration points were used (Figure 3). The LOD was calculated at a signal-to-noise (S/N) ratio of 3 and the LOQ for an S/N at 10. As measured noise, the peak-to-peak (P2P) noise obtained from OpenLab CDS was applied.

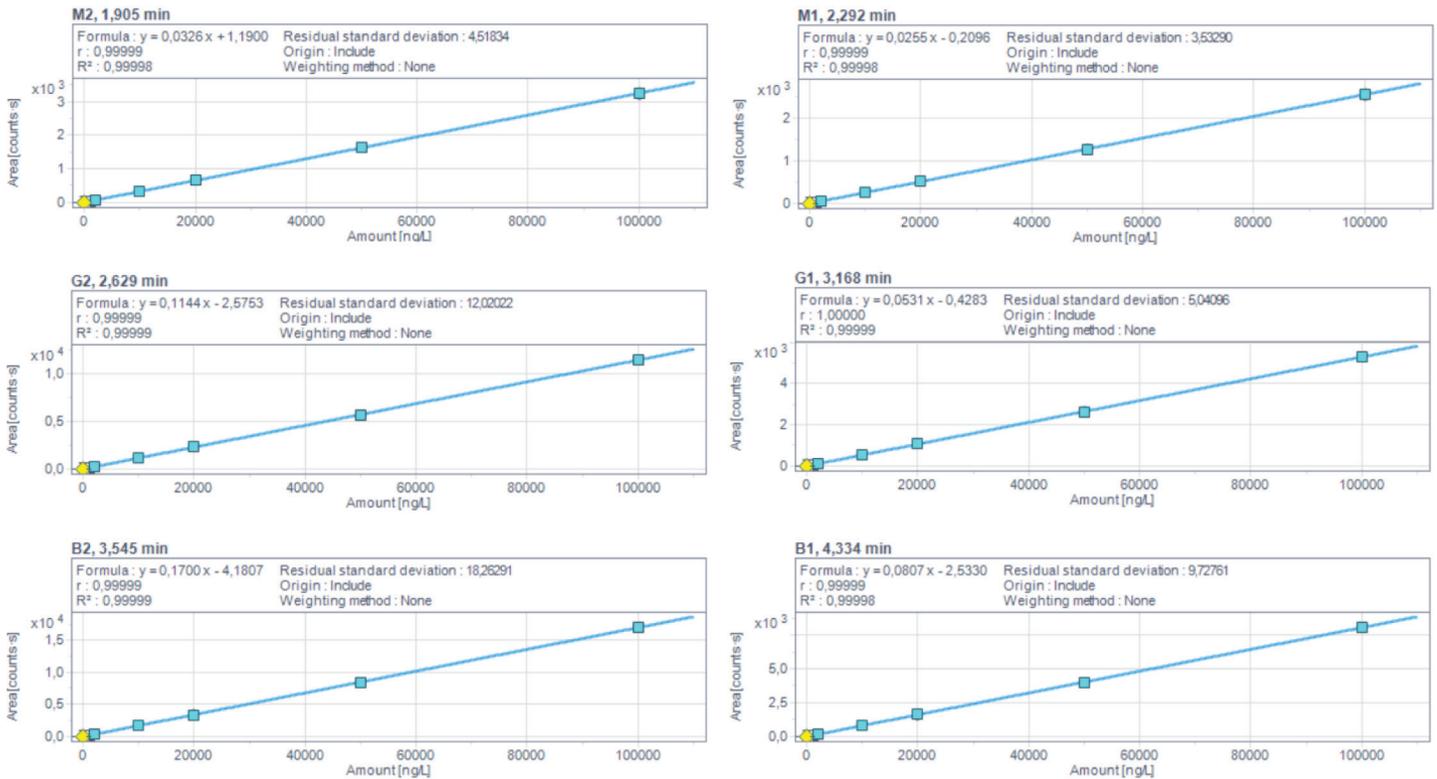


Figure 2. Calibration curves for aflatoxin G2, G1, B2, B1, M2, and M1 in the range of 1 to 100,000 ng/L.

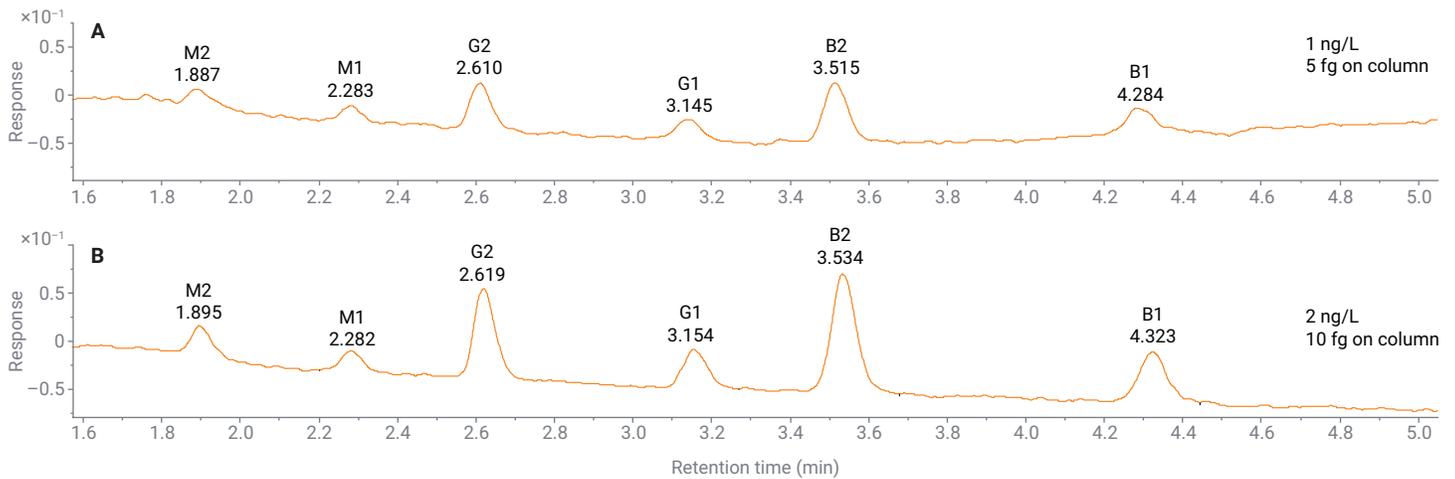


Figure 3. Calibration points at 1 ng/L (A) and 2 ng/L (B).

The lower chromatogram in Figure 3 shows the aflatoxins G1 and M1 in the order of their LOQ (< 2 ng/L) and the upper chromatogram in Figure 3 shows the aflatoxins G2, B1, B2, and M2 in the order of their LOQ (\leq 1 ng/L). The calculated values are summarized in Table 1. Due to the applied injection volume, the chromatogram in Figure 3 (upper) displays the peaks for an amount of 5 fg on column and the lower chromatogram at 10 fg on column for each aflatoxin.

Finally, the 1,000 ng/L calibration point was measured 10 times to calculate peak performance parameters like retention time, area RSDs, peak symmetry, and tailing factor. The retention time RSDs are typically below 0.15% and area RSDs below 0.38% (Table 2).

Conclusion

This application note proves that the Agilent 1290 Infinity III Fluorescence Detector is capable of measuring fluorescent-active aflatoxins without post-column derivatization at high sensitivity down to the single-digit fg/ μ L level. All LODs for the aflatoxins G2, G1, B2, B1, M2, and M1 were below 1 fg/ μ L. Calibrations for quantification over five decades between 1 and 100,000 ng/L showed excellent linearity.

Table 1. Summary of results for linearity, LOQ, and LOD (n = 6).

Aflatoxin	Retention Time (min)	R ²	LOQ (ng/L)	LOD (ng/L)
M2	1.953	0.99998	1.0	0.34
M1	2.377	0.99998	1.8	0.78
G2	2.677	0.99999	0.5	0.15
G1	3.218	0.99999	1.3	0.49
B2	3.637	0.99999	0.4	0.12
B1	4.424	0.99998	0.8	0.25

Table 2. Performance parameters of aflatoxins detected by FLD (n = 10).

Compound	Retention Time RSD (%)	Area RSD (%)	Symmetry	Tailing Factor
M2	0.13	0.35	0.83	1.13
M1	0.12	0.31	0.88	1.09
G2	0.14	0.18	0.89	1.08
G1	0.11	0.31	0.92	1.06
B2	0.11	0.17	0.99	0.99
B1	0.11	0.38	1.04	0.95