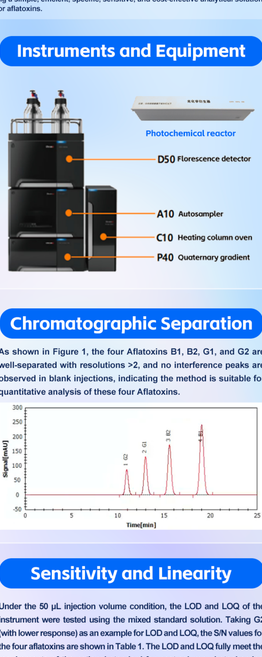
# Determination of Aflatoxins in Foods by Chromai Ultra-High Performance Liquid Chromatography-Fluorescence Detector

Aflatoxins, produced by Aspergillus flavus and A. parasiticus, are class I carcinogens. Among them, aflatoxin B1 (AFB1) is the most toxic, with strong hepatotoxicity and teratogenicity. The current Chinese standard GB 5009.22-2016 specifies the detection methods for aflatoxins in foods, including isotope dilution liquid chromatography-tandem mass spectrometry (Method 1) and high-performance liquid chromatography-fluorescence detection (HPLC-FLD) (Method 2: pre-column derivation, Method 3: post-column derivation).

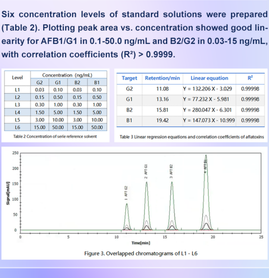
Compared with pre-column derivation and post-column chemical reagent derivation, post-column photochemical derivation eliminates manual sample pretreatment, avoids contamination of the chromatographic system by derivatization reagents, and offers advantages such as simple system configuration and low device cost. This protocol develops a post-column photochemical derivation method for analyzing aflatoxins B1, B2, G1, and G2 using the Chromai Leaps UHPLC-fluorescence detection system, in accordance with Method 3 of GB 5009.22-2016. The method's limits of detection (LOD) and quantitation (LOQ) fully meet the requirements of current national standards, providing a simple, efficient, specific, sensitive, and cost-effective analytical solution for aflatoxins.

## Instruments and Equipment




## Chromatographic Separation

As shown in Figure 1, the four Aflatoxins B1, B2, G1, and G2 are well-separated with resolutions >2, and no interference peaks are observed in blank injections, indicating the method is suitable for quantitative analysis of these four Aflatoxins.




## Sensitivity and Linearity

Under the 50 μL injection volume condition, the LOD and LOQ of the instrument were tested using the mixed standard solution. Taking G2 (with lower response) as an example for LOD and LOQ, the S/N values for the four aflatoxins are shown in Table 1. The LOD and LOQ fully meet the requirements of the national standard for post-column photochemical derivation HPLC (B1/G1 LOQ: 0.1 μg/kg, B2/G2 LOQ: 0.03 μg/kg).

| Target | LOD | | | | LOQ | | | |
|---|---|---|---|---|---|---|---|---|
| | G2 | G1 | B2 | B1 | G2 | G1 | B2 | B1 |
| Density (ng/mL) | **0.015** | 0.05 | 0.015 | 0.05 | **0.03** | 0.1 | 0.03 | 0.1 |
| S/N(p-p) | **3.069** | 4.853 | 5.917 | 8.799 | **10.188** | 12.723 | 18.779 | 26.197 |

Table 1 Sensitivity Test Results

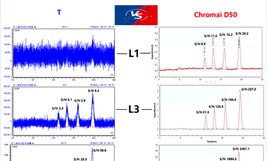



Figure 2 Chromatogram G2 of standard solution A: 0.015 ng/mL, G1: 0.05 ng/mL, B2: 0.015 ng/mL, B1: 0.05 ng/mL (LR); Chromatogram G2 of standard solution B: 0.03 ng/mL, G1: 0.1 ng/mL, B2: 0.03 ng/mL, B1: 0.1 ng/mL (Right)

Six concentration levels of standard solutions were prepared (Table 2). Plotting peak area vs. concentration showed good linearity for AFB1/G1 in 0.1-50.0 ng/mL and B2/G2 in 0.03-15 ng/mL, with correlation coefficients ($R^2$) > 0.9999.

| Level | Concentration (ng/mL) | | | |
|---|---|---|---|---|
| | G2 | G1 | B2 | B1 |
| L1 | 0.03 | 0.10 | 0.03 | 0.10 |
| L2 | 0.15 | 0.50 | 0.15 | 0.50 |
| L3 | 0.30 | 1.00 | 0.30 | 1.00 |
| L4 | 1.50 | 5.00 | 1.50 | 5.00 |
| L5 | 3.00 | 10.00 | 3.00 | 10.00 |
| L6 | 15.00 | 50.00 | 15.00 | 50.00 |

Table 2 Concentration of serie reference solvent

| Target | Retention/min | Linear equation | $R^2$ |
|---|---|---|---|
| G2 | 11.08 | Y = 132.206 X - 3.029 | 0.99998 |
| G1 | 11.16 | Y = 77.232 X - 5.981 | 0.99996 |
| B2 | 15.81 | Y = 280.047 X - 6.301 | 0.99998 |
| B1 | 19.42 | Y = 147.073 X - 10.999 | 0.99998 |

Table 3 Linear regression equations and correlation coefficients of aflatoxins

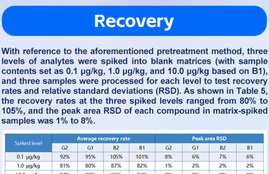



Figure 3. Overlapped chromatograms of L1 - L6

## Sensitivity Comparison

Under the same experimental conditions, the sensitivity of the Chromai Leaps UHPLC-fluorescence system was compared with imported Brand T. Using identical mobile phase, column, and standards, results showed that at the lowest standard level (L1), Brand T's detector did not show peaks, while Chromai's detector had significantly higher sensitivity at the same concentrations (L3, L5) (Figure 4).




Figure 4. Comparison of the sensitivity between Chromai D50 and Thermo Fisher (at three concentration levels of L1, L3 and L5)

## Repeatability

Three concentration levels (L1, L3, L6) were injected 6 times each. Retention time RSD ≤ 0.2%, and peak area RSD < 5% (Table 4).

| Concentration level | Retention time RSD | | | | Peak area RSD | | | |
|---|---|---|---|---|---|---|---|---|
| | G2 | G1 | B2 | B1 | G2 | G1 | B2 | B1 |
| L1 | 0.2% | 0.2% | 0.2% | 0.2% | 4.7% | 4.6% | 3.9% | 3.4% |
| L3 | 0.1% | 0.1% | 0.1% | 0.1% | 1.6% | 1.5% | 1.2% | 0.9% |
| L6 | 0.1% | 0.1% | 0.1% | 0.2% | 0.6% | 0.7% | 0.7% | 0.9% |

Table 4. Examination of repeatability

## Recovery

With reference to the aforementioned pretreatment method, three levels of analytes were spiked into blank matrices (with sample contents set as 0.1 μg/kg, 1.0 μg/kg, and 10.0 μg/kg based on B1), and three samples were processed for each level to test recovery rates and relative standard deviations (RSD). As shown in Table 5, the recovery rates at the three spiked levels ranged from 80% to 105%, and the peak area RSD of each compound in matrix-spiked samples was 1% to 8%.

| Spiked level | Average recovery rate | | | | Peak area RSD | | | |
|---|---|---|---|---|---|---|---|---|
| | G2 | G1 | B2 | B1 | G2 | G1 | B2 | B1 |
| 0.1 μg/kg | 92% | 95% | 105% | 101% | 8% | 6% | 7% | 6% |
| 1.0 μg/kg | 81% | 80% | 87% | 82% | 1% | 2% | 2% | 2% |
| 10.0 μg/kg | 83% | 89% | 95% | 94% | 7% | 8% | 8% | 8% |

Table 5. Recovery rate and repeatability

## Sample Testing

The homemade sesame oil sample showed trace detection of B1 and B2, both below the method's LOQ, with estimated contents of 0.015 and 0.004 μg/kg, respectively (Figure 5).




Figure 5. Overlapped chromatograms of the sample, solvent blank and standard substance

## Conclusion

This application demonstrates a method for simultaneous determination of aflatoxins B1, B2, G1, and G2 in foods using Chromai UHPLC-fluorescence detector with post-column photochemical derivatization. The method features good linearity, reproducibility, and sensitivity, with LOD/LOQ meeting national standard requirements. Compared to other derivatization methods, photochemical derivatization offers simpler equipment, lower cost, and avoids chemical reagent damage or manual operation risks, making it a superior solution for aflatoxin analysis.