Aflatoxin Immunoaffinity Chromatography with Automated Vacuum Manifold

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INTRODUCTION

Mycotoxins are heat-stable toxic metabolites produced by fungi that persist through food processing and pose significant health risks due to their accumulation in fatty tissues. Aflatoxins are potent carcinogens commonly found in agricultural commodities such as corn and peanuts, necessitating reliable analytical methods for regulatory compliance. AOAC Official Method 991.31 utilizes immunoaffinity column cleanup for selective aflatoxin extraction. However, this approach is traditionally manual, labor-intensive, and dependent on precise flow control, leading to inefficiencies and potential variability in high-throughput laboratory settings.

In this study, an automated workflow using the Extraction+ connected device vacuum manifold and OneLab™ software was developed to modernize aflatoxin analysis. Pressure gradients were optimized to maintain controlled flow rates and ensure consistent sample processing across challenging matrices. The method demonstrated excellent linearity ($R^2 \geq 0.995$), reproducibility (<10% RSD) over a range of 0.1–100 ng/g in corn, and accurate recoveries (95–110%) for aflatoxins B1, B2, G1, and G2. These results show that Extraction+ connected device provides a robust, efficient, and reproducible alternative to traditional positive pressure methods while maintaining the analytical performance of the AOAC protocol. Integration of OneLab software with LIMS and Empower™ software enables full end-to-end traceability, automated data handling, and reduced manual intervention.

EXPERIMENTAL

Following AOAC 991.31, 25 g of ground corn (Trilogy RM, p/n TR-A1000) was blended with 5 g NaCl and 125 mL 70% methanol at high speed for 2 minutes and gravity filtered through fluted filter paper (Waters VICAM™, p/n 31240, Milford, MA). 15 mL of filtrate was diluted with 30 mL water, mixed well and gravity filtered through a microfiber filter (Waters VICAM, p/n 31955). 15 mL of the filtrate was then loaded on an AflaTest™ IA cartridge (Waters VICAM, p/n 205002642 (G1010)). In the three-step cleanup, 15 mL of the now twice-filtered sample was loaded, washed twice with 10 mL aliquots of water and eluted with 1.0 mL methanol. Eluates were diluted 1:1 with water and directly injected into an ACQUITY™ UPLC™ H-Class PLUS System paired with an ACQUITY UPLC FLR Detector. The sample preparation procedure for AOAC 991.31 is summarized in **Figure 1**.

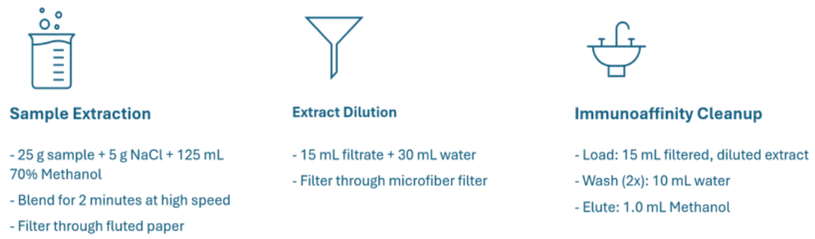

Figure 1: Representation of the sample preparation protocol for AOAC Official Method 991.31 Aflatoxins in Corn, Raw Peanuts, and Peanut Butter: Immunoaffinity Column (AflaTest) Method

LC Conditions:

System: ACQUITY H Class Plus System with FTN
Column: ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm (Waters, p/n 186003539)
Column Temperature: Room Temperature
Mobile phase: 45% methanol, v/v
Flow: 0.350 mL/min
Run time: 6 minutes
Injection volume: 5 µL

Detector Conditions:

System: ACQUITY UPLC FLR Detector
Excitation: 360 nm
Emission: 440 nm
Data units: Emission
Data rate: 10 pts/sec
PMT gain: 1.00
Time: 0.2 sec
Software: Empower software (Ver. 3.6.1)

Automation and Smart Devices:

Extraction+™ and Pipette+™ Connected Devices driven by OneLab™ Software

RESULTS

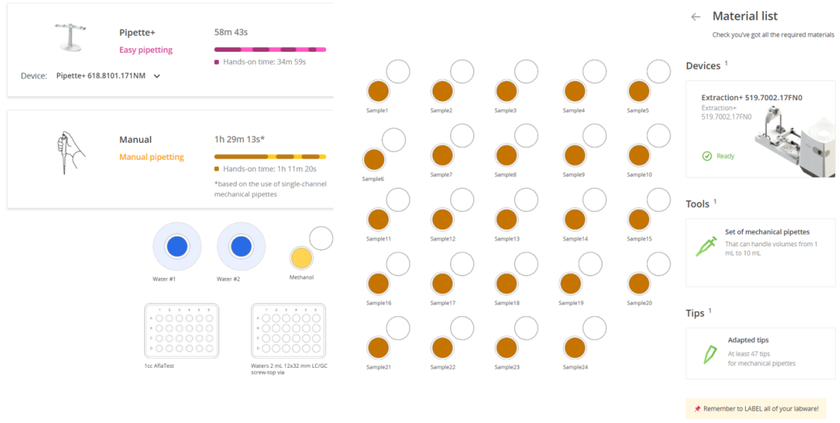

Figure 3: OneLab software protocols for assisted or manual pipetting using existing lab tools or connected devices

Figure 3: Compact setup allows cleanup of 24 samples at a time. A volume extension barrel is coupled to the cartridge to allow the entire sample to be loaded in a single step

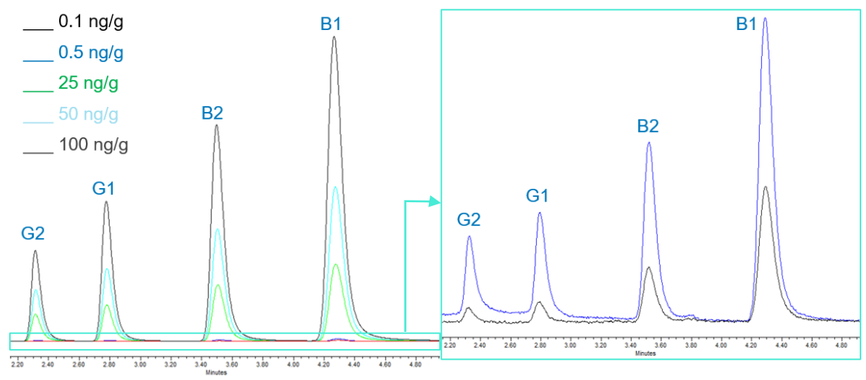

Figure 4: Chromatograms showing the separation of aflatoxins G2, G1, B2, and B1 with a C18 column using a 6-minute isocratic flow of 45% methanol at a rate of 0.350 mL/minute and fluorescence detection

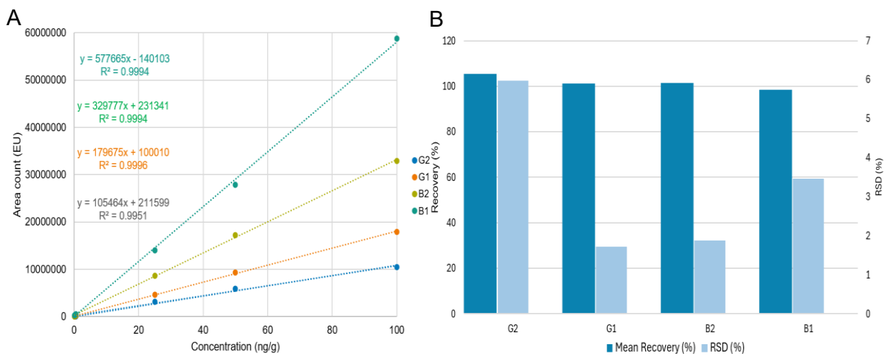

Figure 5: Calibration curves showing extraction linearity and reproducibility for aflatoxins G2, G1, B2, and B1 across a concentration range of 0.1–100 ng/g (A). Mean recoveries and RSDs for the four analytes (B)

DISCUSSION

Extraction+ connected device effectively handles difficult sample matrices through controlled vacuum pressure gradients, ensuring consistent flow and preventing clogging while maintaining optimal elution rates. When paired with a OneLab software workflow, the system delivers accurate, reproducible aflatoxin quantification comparable to traditional methods and can replace positive pressure pumps using only a fraction of its capacity. Integration with LIMS and Empower software further enhances efficiency by enabling seamless sample tracking, automated data transfer, and reduced risk of manual errors across the entire analytical process.

We have demonstrated that the AflaTest Column sample preparation method may be carried out using an Extraction+ Connected Device vacuum manifold with Easy pipetting using Pipette+ Automated Pipetting. This reduces the need for manual intervention, reducing the amount of hands-on time by 50% and provides for high recoveries and excellent reproducibility. This was demonstrated for corn samples that were spiked with aflatoxins B1, B2, G1 and G2 at concentrations ranging from 0.1 - 100 ng/g. Using OneLab software integrated with a LIMS and Empower software further enhances efficiency by enabling seamless sample tracking, automated data transfer, and reduced risk of manual errors across the entire analytical process. Together, these benefits offer an attractive alternative to manual AflaTest sample preparation that should be particularly beneficial for labs that carry out many of these tests.

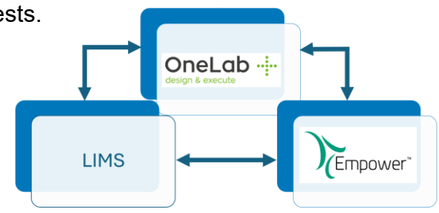

Figure 6: Integrated LIMS, OneLab software, and Empower software workflows ensure compliant chain of custody, automated sample management, and secure, and seamless data management

CONCLUSIONS

- High throughput capability: Extraction+ connected device processes up to 24 samples simultaneously in a compact footprint, reducing the processing time by 50% compared to manual sample preparation
- Increased reproducibility: automated, programmable pressure gradients minimize operator-to-operator variability and maintain consistent AflaTest cartridge loading and elution
- Maintained AOAC-level selectivity: AflaTest immunoaffinity cleanup remains fully compliant with AOAC 991.31 performance expectations
- Robust performance: controlled vacuum gradients prevent clogging and maintain flow even with viscous or particulate-rich food matrices
- Excellent analytical performance: demonstrated linearity and precision for aflatoxins B1, B2, G1, and G2.
- Full digital traceability: LIMS + OneLab software + Empower software integration ensures chain of custody, automated sample lists, and seamless data transfer with the AflaTest workflow

References

1. AOAC, Aflatoxins in Corn, Raw Peanuts, and Peanut Butter, Immunoaffinity Column (AflaTest) Method 991.31, (2005). AOAC INTERNATIONAL.
2. Sun, T.; Dreolin, N.; Hird, S.; and Collette N. Analysis of Aflatoxins in Corn and Peanuts Using Immunoaffinity Chromatography and the Arc™ HPLC System, (2023). Waters Application Note: 720007843.
3. Benvenuti, M.E.; and Di Gioia , A.J., Rapid Analysis of Aflatoxins without Derivatization Using Ultra Performance Liquid Chromatography and Fluorescence Detection, (2019), Waters Application Note: 720002644.