Performance Validation Study of the Detection of Aflatoxin in Pistachios and Almonds Using an Immunoaffinity Column Coupled with HPLC or Fluorometry

Alex Kostin, Chris Roebuck and Tony Lupo

NeoColumn Aflatoxin

Sample Preparation and Extraction

1. Grind sample to a fine particle size. Mix well.
2. Mix in a blender jar for 2 minutes:
   • 25 g ground sample
   • 5 g salt (NaCl)
   • 125 mL 60% MeOH
3. Filter extract through pleated filter paper.
4. Dilute 1:1 using 10 mL of filtered extract with 10 mL distilled water.
5. Filter dilute through glass microfiber filter paper.
6. Sample is now ready for testing.

INTRODUCTION

Deriving from the molds Aspergillus flavus and A. parasiticus, aflatoxin is a mycotoxin that is known to be very harmful to animals and humans. Excessive ingestion has been associated with liver damage, immune suppression and even cancer. There are four principle types of aflatoxin (B1, G1, G2, and B2) with the most toxic and most frequently encountered of the group being B1. The toxin can be found most widely in corn, peanuts and the majority of tree nuts and currently is regulated by the FDA and cannot exceed 20 ppb. Testing for the presence of aflatoxin in nuts is important to the safety of food and feed. There are many methods used for the detection of total aflatoxin in various commodities. Other methods may not allow for differentiation between aflatoxin types. However, an immunoaffinity column separation coupled with fluorometry (to determine total aflatoxin) or with HPLC (to quantify aflatoxin by its individual components) provides a more precise method.

EXTRACTION METHODS AND PRINCIPLES

Obtained from the AOCS Proficiency Program, a total of 4 blind-coded samples of almond paste and 4 blind-coded samples of pistachio paste were extracted and prepared for analysis on both HPLC and direct read fluorometry. These sample extracts were analyzed via the test procedure outlined below:

- The top cap was removed and the bottom plug of the column was loosened and attach to the syringe reservoir.
- A 15 mL volume of the prepared extract was added to the reservoir. The bottom plug was removed and pressure was applied to the reservoir to initiate flow at a rate of approximately 2 drops/second. Flow was continued until the column ran dry.
- A 10 mL volume of distilled water was added to the reservoir and pressure was applied to the reservoir to initiate flow at a rate of approximately 2 drops/second. Pressure was continued until the column ran dry. This step was repeated with a 20 mL wash.
- A cuvette was placed under the column and 1 mL of 100% methanol was applied to the syringe reservoir. Flow was initiated by applying pressure at a rate of approximately 2 drops/second. This was continued until the column ran dry.
- For quantification using direct read fluorometry, a 1 mL volume of prepared developer was added directly to the cuvette. This was mixed by vortexing and used to calibrate fluorometer.
- For quantification using HPLC, the eluate was diluted using 1 mL HPLC grade water and analyzed by HPLC was completed using reference method 991.31.

CONCLUSION

Upon completion of the AOCS Proficiency Program, AOCS sent out actual values for all blind samples. Based on those official results, recoveries and CVs were calculated. Aflatoxin was accurately recoverable in both pistachios and almonds. In pistachios, aflatoxin was recovered at 104.88% with a CV of 6.80%. In almonds, the recovery of aflatoxin was 106.59% with a CV of 7.33%. When using HPLC as the method of determination, aflatoxin recovery was 111.16% in pistachios and 112.71% in almonds with CVs of 2.32% and 2.25% respectively.

The recoveries are equally impressive when breaking down the individual types of aflatoxin in pistachios and almonds. B1 was recovered at 104.28% and 110.39% respectively, B2 at 95.17% and 94.50%, G1 at 100.19% and 106.33% and G2 was non-detected for both commodities. These data demonstrate that Neogen’s NeoColumn for Aflatoxin provides a robust and accurate method for detection of aflatoxin in nut-based commodities.

ABSTRACT

Neogen Corporation has developed, in conjunction with AOAC Official Method 991.31, the NeoColumn for Aflatoxin DR for the detection of total aflatoxin using direct read fluorometry. The NeoColumn was also developed to detect aflatoxin B1, G1, G2, and G3 using high performance liquid chromatography (HPLC). The purpose of this study was to evaluate the method for accuracy and precision on tree nuts. Internal studies at Neogen Corporation showed the NeoColumn method coupled with direct read fluorometry had a 104.9% relative recovery of the reference value in pistachios with a CV of 2.3%. When used with HPLC it demonstrated 111.2% relative recovery of the reference value in pistachios with a CV of 2.3%. The NeoColumn method coupled with direct read fluorometry revealed 106.6% relative recovery of the reference value in almonds with a CV of 6.8% and when coupled with HPLC demonstrated 112.71% relative recovery with a CV of 2.3% across all samples analyzed. When analyzed for the four main types of aflatoxin (B1, G1, G2, and G3) the recovery for each was found to be exceptional. This method proves effective for use with tree nuts in addition to the previously validated commodities of peanuts and corn.

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- A 15 mL volume of the prepared extract was added to the reservoir. The bottom plug was removed and pressure was applied to the reservoir to initiate flow at a rate of approximately 2 drops/second. Flow was continued until the column ran dry.
- A 10 mL volume of distilled water was added to the reservoir and pressure was applied to the reservoir to initiate flow at a rate of approximately 2 drops/second. Pressure was continued until the column ran dry. This step was repeated with a 20 mL wash.
- A cuvette was placed under the column and 1 mL of 100% methanol was applied to the syringe reservoir. Flow was initiated by applying pressure at a rate of approximately 2 drops/second. This was continued until the column ran dry.
- For quantification using direct read fluorometry, a 1 mL volume of prepared developer was added directly to the cuvette. This was mixed by vortexing and used to calibrate fluorometer.
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