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The HPLC Analysis of Aflatoxins in Raw Peanuts with Sep-pak® Cleanup

W. Jeffrey Hurst* Kevin P. Snyder and Robert A. Martin, Jr.

ABSTRACT

An HPLC method for the determination of aflatoxins is described. The aflatoxins are extracted with aqueous acetone and interfering compounds precipitated with $CuCO_3$. After defatting, the aflatoxins are extracted into CH_2Cl_2 for cleanup with silica Sep-pak® which eliminates other interfering compounds. The resulting extract is then treated with TFA to form the hemiacetal derivatives prior to final HPLC analysis. Data indicated that the method exhibits excellent accuracy and precision. In addition, it is time and solvent conservative.

Key Words: HPLC, aflatoxin, raw peanuts.

Initial use of HPLC in aflatoxin analysis is centered on its use as a final determination step since it produced accurate and precise data. However, earlier methodology did not address the concerns of sample preparation for subsequent HPLC analysis (1,2,3,4). Current HPLC methods for the determination of aflatoxins in peanuts require rather rigorous cleanup procedures for the removal of interfering materials (1,2,3,4). Additionally, the time required and solvent consumed is still comparable to the TLC methods. There is a need for rapid, accurate and sensitive methods for this assay. The assay outlined here decreases solvent usage and is suitable for raw peanuts with limits of detection that compare favorably with other methods.

Materials and Methods

Apparatus

The LC system used consisted of a Waters 6000A Pump, Waters WISP 710B Autoinjector, and a Gilson Spectraglo Fluorometer. The HPLC column was 3.9 mm x 30 cm packed with 10 μm Spherisorb ODS (by HPLC Technology Inc.).

Chemicals and Reagents

Acetonitrile and methanol were HPLC grade; the other solvents/chemicals were ACS grade. The HPLC mobile phase was 62/18/18 (v/v/v) ($H_2O/CH_3CN/CH_3OH$) at a flow rate of 2.2 mL/min.

Assay Procedure

A 50-g sample of raw peanuts was placed in a quart Waring Blender along with 5-g of $CuCO_3$ and 250 mL of 85/15 (v/v) (acetone/water). This mixture was blended at low speed for 1 min. to alleviate bumping and then blended at high speed for 3 min. Alter blending, the mixture was filtered through Whatman 2v or equivalent. A 50-mL aliquot was collected and placed in a 250-mL separatory funnel which contained 50-mL of 10% NaCl (w/v) and 30 mL of hexane. This was shaken for 1 min. and the hexane discarded. An additional 30-mL aliquot of hexane was added and the procedure was repeated. The remaining aqueous layer was partitioned with two 35-mL portions of dichloromethane. The dichloromethane aliquots were combined and placed in a 100-mL round bottom flask and rotary evaporated under vacuum at 35 C. Ten mL of CH_2Cl_2 is then added to the residue prior to Sep-pak® treatment.

Sep-pak® Cleanup

Prior to introduction of the sample, a silica Sep-pak® was conditioned with 5-mL of hexane. A 5-mL dichloromethane aliquot of the aflatoxin containing residue was then run through the Sep-pak®. The aflatoxins were eluted with 3-mL of 95/5 (v/v) $CHCl_3$/EtOH and taken to dryness using low heat and a stream of nitrogen.

Derivative Formation

Seventy-five μL of 80% trifluoracetic acid (TFA) was added to the sample and allowed to react for 30 seconds. The TFA was removed under low heat with a stream of nitrogen. After cooling the residue was brouhgt up to a known volume (250-300 μL) in mobile phase prior to analysis. Injection volume was typically 25-50 μL, depending on concentration.

Results and Discussion

Figures 2,3,4, and 5 are chromatograms of the derivatized aflatoxin standard, a raw peanut extract spiked with aflatoxin. An unspiked raw peanut extract, and an extract that was injected with no sample cleanup.

In this communication, the use of aflatoxin derivatives was used since it serves many purposes. In earlier HPLC assays for aflatoxin, the analyst was required to use two detectors, UV at 365 nm for aflatoxins B_1 and G_1, and Fluorescense for aflatoxins B_2 and G_2 if in the normal phase mode. This method eliminates the use at dual detectors, since in the reversed phase mode the derivatives for aflatoxin B_1 and G_1 exhibit excellent fluorescence. Aflatoxins B_1 and G_1 are converted to aflatoxin B_{2_a} and G_{2_a} by an acid catalyzed addition of water to the vinyl ether double bonds of aflatoxin B_1 and G_1, producing a hemiacetal as seen in Figure 1. The structure of

Hershey Foods Corporation, Analytical Research Group, 1025 Reese Avenue, P. O. Box 805, Hershey, PA 17033-0805.

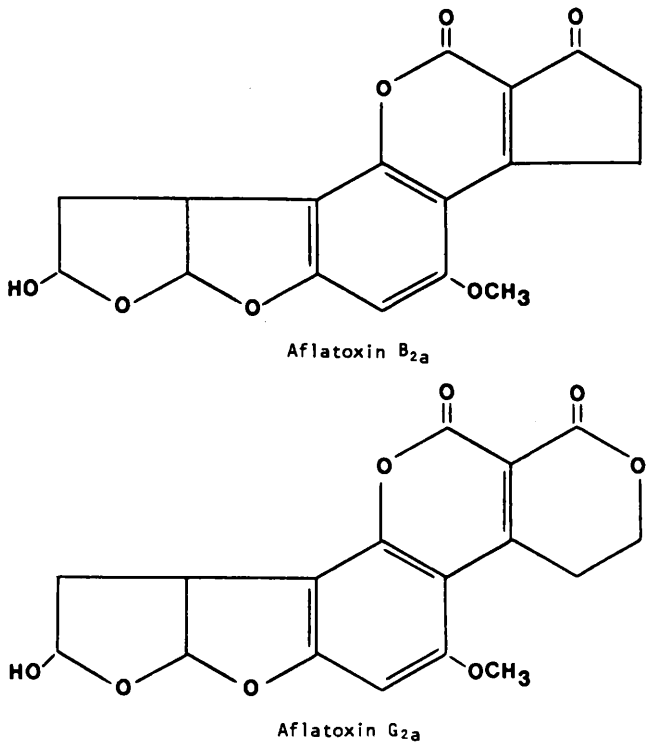

Fig. 1. Structure of alfatoxins B_{2_a} and G_{2_a}.

these new compounds was confirmed by PMR, IR and MS and shown to be identical to the compounds isolated by Dutton and Heathcote (5,6). Since that time the formation of these derivatives has been used as confirmation of aflatoxin B_1 and G_1. In this reaction, aflatoxins B_2 and G_2 are unchanged.

Another benefit realized is that sample extracts produced negligible background interferences as can be seen in Figures 2 and 3 when compared to UV traces. Additionally, one is able to achieve lower limits of 2-5 ppb total aflatoxin with good accuracy and precision.

Standard and sample precision studies are summarized in Tables 1 and 2, while recovery studies are summarized in Table 3.

Table 1. Standard precision studies (n = 5).

Toxin	Conc (ng)	% Cv
B_{2_a}	2.5	5.6
G_{2_a}	2.5	5.7
B_2	0.75	5.6
G_2	0.75	8.1

Table 2. Sample precision studies (n = 5).

Toxin	∿Conc (ng)	% Cv
B_{2_a}	2.5	2.2
G_{2_a}	2.5	5.2
B_2	0.75	3.4
G_2	0.75	4.1

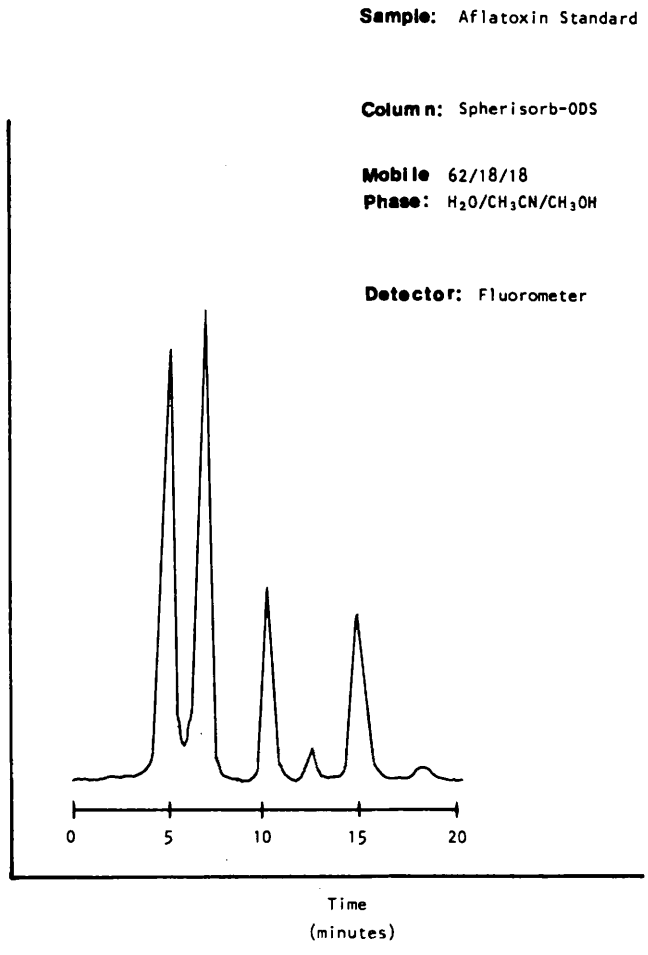

Fig. 2. Chromatogram of aflatoxin standard.

Table 3. Recovery studies (n = 2).

Toxin	Conc (ng)		Recovery	
B_{2_a}	10	20	103	95.8
G_{2_a}	10	20	100	93
B_2	3	6	98	89
G_2	3	6	98	89

Table 1 summarizes precision studies conducted with aflatoxin standards with five determinations of each. Concentrations ranged from 0.75 ng of aflatoxin B_2 and G_2 to 2.5 ng of toxins B_1 and G_1. The coefficients of variation ranged from 5.6 to 8.1%.

Table 2 summarizes similar data for samples containing the four aflatoxins. Coefficients of variation ranged from 2.2 to 5.2%. The third table outlines the recovery study based on duplicate determinations at two levels of added aflatoxin. Recoveries ranged from a low of 89% to a high of 103%.

These results indicate the the proposed method provides an accurate and precise method for the determination of aflatoxins in peanuts. In addition to being accurate and precise, the method is attractive in the area of time savings. The method can be performed in approximately 25-30 minutes depending on the analyst. It additionally provides a substantial decrease in solvent usage,

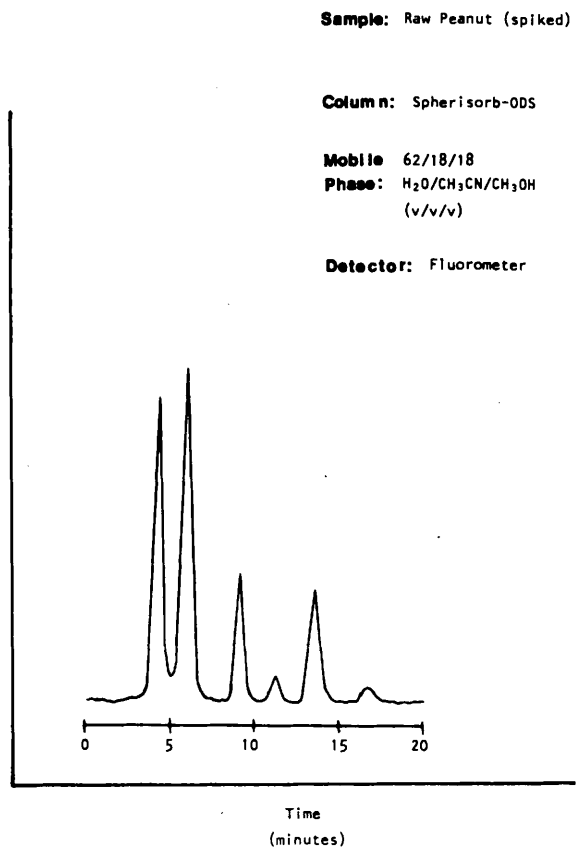

Fig. 3. Chromatogram of artificially contaminated peanut extract spiked.

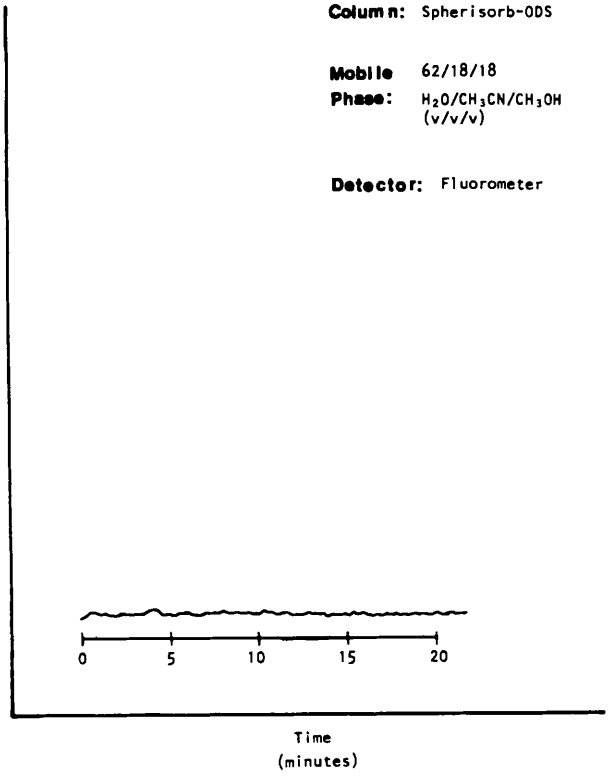

Fig. 4. Chromatogram of peanut extract.

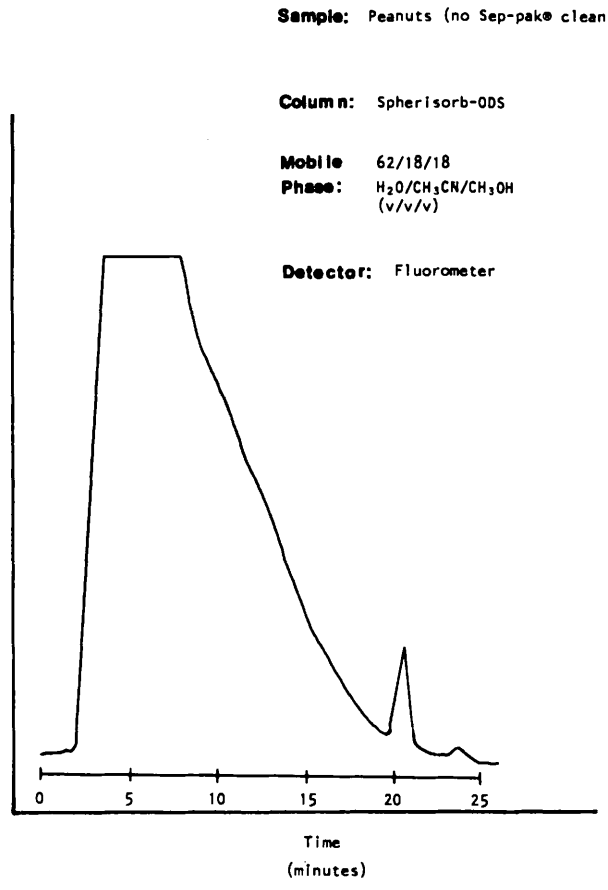

Fig. 5. Chromatogram of peanut extract with no Sep-pak® cleanup.

Therefore allowing cost savings and decreased exposure to solvent vapors. The cleanup column used in other assays is now replaced with a disposable cartridge, thereby increasing sample to sample extraction consistency by elimination of the individual packing of columns. The use of the hemiacetal derivatives increases sensitivity several fold. With the emphasis in productivity in all areas, this technique will allow for accelerated sample thruput with increased accuracy and precision.

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