Rapid Method for Detecting Aflatoxins in Peanuts

CHARLES E. HOLADAY,[1] Peanut Quality Investigation, Albany, Georgia 31702

Abstract

The thin-layer chromatographic procedure for detecting and quantifying aflatoxin in peanuts is too time-consuming, costly, and difficult to be practical for use by untrained personnel. A method based on millicolumn chromatography was tested and found to be both rapid and simple. Columns are prepared by filling a length of 4-mm glass tubing with silica gel to a depth of about 4.5 cm. The millicolumn is developed in a chloroform-methanol extract of a peanut sample. If aflatoxin is present, a blue fluorescent band at the lower end of the column is observed when the column is exposed to long-wave ultraviolet radiation. Sensitivity is in the order of 5 ppb and an assay can be completed in 15–25 min. Some degree of quantification is possible by comparison with columns developed in extracts with known aflatoxin contents.

Introduction

THE ESTIMATION OF AFLATOXIN in peanuts is based on analytical procedures utilizing thin-layer chromatography (TLC) of partially purified extracts (2,3). Chemical clean-up of the extracts and interpretation of the developed plates require expensive laboratory equipment and trained personnel. An assay may require 2 hr or longer, depending on the amount of clean-up. These procedures, therefore, are not practical for use by peanut graders or other workers who have little or no training in chemical methods.

The present study was undertaken to explore other procedures which might be adapted to a simple and rapid assay method for peanuts. Colorimetric methods which were investigated did not have the required sensitivity, and the clean-up was as time-consuming and as difficult as that for thin-layer chromatography. Solution fluorescence also proved to be impractical because other material extracted from peanuts interfered with the aflatoxin fluorescence.

The method presented in this paper is based on millicolumn chromatography (MCC) in which the millicolumn is developed in a chloroform-methanol extract of peanuts. The column is removed and exposed to long-wave ultraviolet radiation. If aflatoxin is present, a blue fluorescent band about 10 mm from the lower end of the column is visible.

This method is similar to column chromatography but is on a smaller scale. A 4-mm (ID) glass tube is used and the column is 4.5 cm long. Ascending development is used. Millicolumn chromatography is faster and simpler than TLC, requiring little training and laboratory equipment.

Method

Preparation of the millicolumn

The millicolumn is prepared by plugging one end of a piece of 4-mm glass tubing with glass fiber filter paper. The plug should be about 5 mm long when packed firmly in the tube. A 21 mm circle of glass fiber filter paper provides the correct thickness. Thicker plugs slow development too much and with thinner plugs development proceeds too rapidly and bands are indistinct. Development should require a minimum of 5 min. The column is filled with silica-gel for column chromatography type, 0.05–0.2 mm Brinkmann. After tapping several times to pack, the column should be about 4.5 cm long. A second plug is inserted in the top of the tube and forced down against the silica-gel. The column is then equilibrated in an atmosphere of 78–80% relative humidity; a minimum of 24 hr is required for complete equilibration. A desiccator containing a saturated ammonium chloride solution makes a convenient equilibration and storage chamber. With practice, a technician can make 400–450 columns in an 8-hr day. Fig. 1 shows the preparation of a millicolumn. Fig. 2 shows 2 completed millicolumns using different lengths of tubing.

Extraction

The solvent used for extraction in the millicolumn chromatography procedure contained 97 parts chloroform, 3 parts methanol (v:v); 100 ml of this solvent is used to extract 50 g of peanuts or 200 ml may be used to extract 100 g of peanuts. If the peanuts have a moisture content of above 9%, some emulsification takes place during extraction. The peanuts are extracted in a Waring Blendor operated at high speed for 1 min. After extraction the ground mass is vacuum filtered on a Buchner funnel (size 2 A) using a 9-cm circle of glass fiber filter paper. A 250-ml or larger filter flask may be used to collect the filtrate. After filtration the filtrate is agitated by whirling in the filter flask for 30 sec.

Development

Before developing, the millicolumn should be preconditioned in an atmosphere of 78–80% relative humidity for a minimum of 24 hr. It is then tapped several times and the upper plug forced down firmly against the top of the column to tightly pack the silica-gel. The column is placed in the filtrate and allowed to develop to the top of the column. Development usually requires 10–15 min. It is unnecessary to have an exact amount of filtrate in the filter flask. Enough to cover about 5 mm of the glass tube when it is resting on the bottom of the filter flask is satisfactory.

Detection

After development the column is exposed to long-wave ultraviolet radiation while being warmed at approximately 60C for 5 min. The aflatoxin band is readily seen about 10 mm from the bottom of the column. Individual aflatoxins do not separate; all are present in the single band. A strong source of long-wave ultraviolet radiation is necessary; we used Chromato-vue chamber which provides both the radiation and heat. A thermostat placed on top of the Chromato-vue transilluminator plate and wired across the switch is a convenient means to control the temperature at 60C (Fig. 3).

Experimental Procedures and Discussion

Lee et al. (1) showed that aflatoxin in a contaminated peanut kernel is distributed throughout

[1] Field Crops & Animal Products Research Branch, Market Quality Research Branch, ARS, USDA, Albany, Georgia.

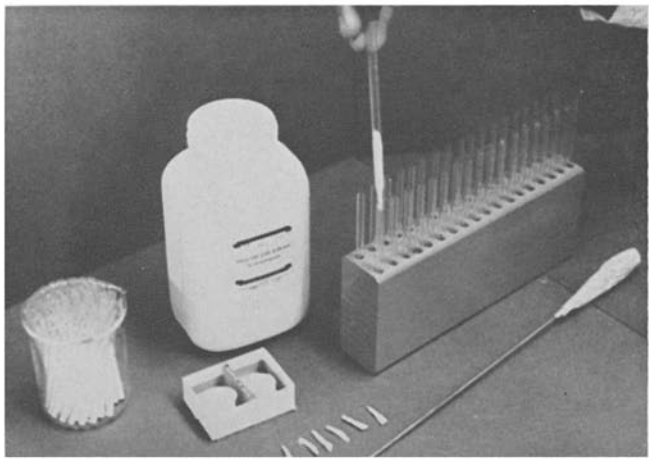

FIG. 1. Preparation of a millicolumn.

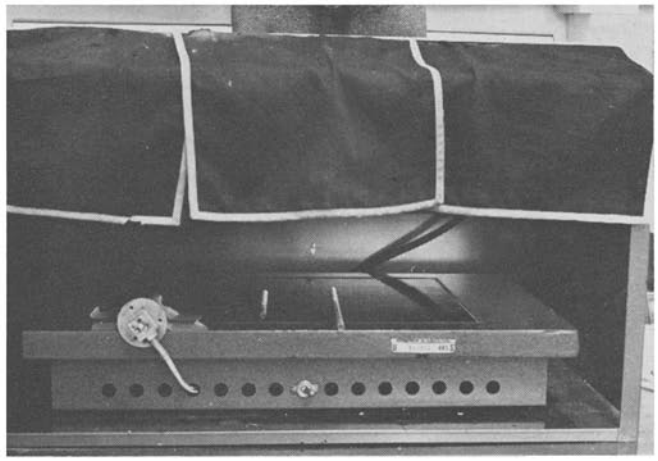

FIG. 3. Chromato-vue chamber showing transilluminator plate with thermostat.

the cotyledons. The extraction of whole or half kernels by shaking with a solvent, therefore, will not remove all of the aflatoxin. Peanuts should be extracted in a Waring Blendor or other similar blender for complete removal of the aflatoxin. Table I shows the results of successive extractions of whole and split peanut kernels in a reciprocal shaker and in a Waring Blendor.

These data were obtained as follows: 100 g of peanuts were shaken with 200 ml of a mixture of 65 parts acetone and 35 parts hexane (v:v) for 10 ml in a reciprocal shaker operating at 280 strokes/min. The peanuts were washed with three 50-ml portions of the acetone-hexane mixture. The washings were added to the filtrate which was assayed for aflatoxin content by a TLC procedure which was a modification of the one developed by Robertson et al. (3). The modification involved changing the extracting solvent system from acetone-hexane-water (50:48.5:1.5 v/v) to acetone-hexane (65:35 v/v). The original 100 g of peanuts were again extracted on the reciprocal shaker for 20 min and washed in the same manner as before. The filtrate was assayed for aflatoxin content. These same peanuts were extracted once more in a Waring Blendor for 1 min and filtered. The peanuts were washed with three 50-ml portions of the acetone-hexane mixture and the washings added to the filtrate. The filtrate was assayed for aflatoxin content. Surprisingly, more aflatoxin was recovered from whole than from half kernels by shaking.

Figure 4 shows 5 developed tubes representing 30, 60, 90, 150, and 250 ppb of aflatoxin, respectively, from left to right. Occasionally materials other than aflatoxin will form a fluorescent background and, in some cases, a partial band immediately after development, but under the influence of the radiation and heat (60C) these interfering materials dissipate in about 5 min, while the aflatoxin bands remain relatively stable for 20 min or longer. Sensitivity is about 5 ppb. Some degree of quantification is possible by comparing columns developed in extracts with unknown aflatoxin contents, with those developed in standard extracts. As an example, if an unknown column has higher fluorescence than the 30 ppb standard column, but lower fluorescence than the 50 ppb standard column, it must have an aflatoxin content between 30 and 50 ppb. Table II illustrates this method of quantification. Four standard columns were developed in extracts containing 30, 50, 100, and 150 ppb, respectively. This method is not as

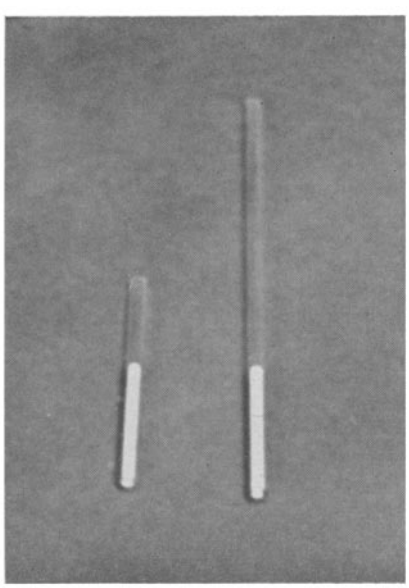

FIG. 2. Two completed millicolumns using different lengths of tubing.

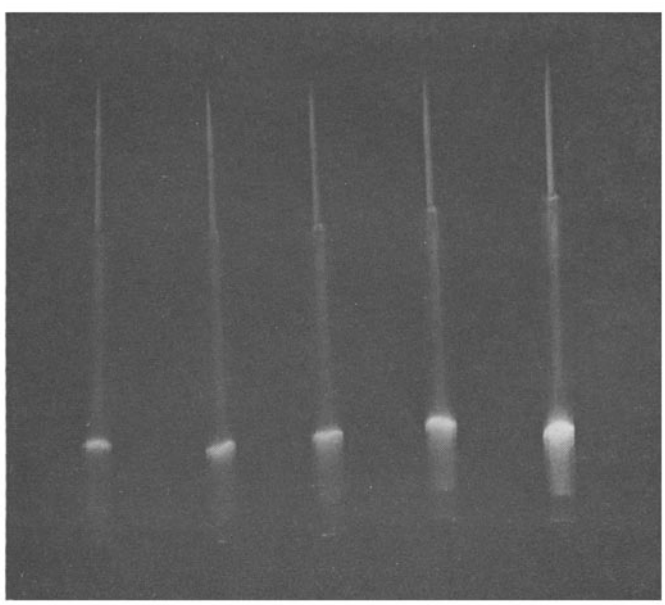

FIG. 4. Five developed millicolumns representing 30, 60, 90, 150 and 250 ppb of aflatoxin, respectively, from left to right.

TABLE I

Results of Successive Extractions of Whole and Split Peanut Kernels in a Reciprocal Shaker and in a Waring Blendor

Sample No.	Shaker		Waring blendor	Accumulative	Recovery after shaking	
	10 min ppb	20 min ppb	1 min ppb	Total ppb	10 min %	30 min (10 + 20) %
Whole Kernels[a]						
1	13	17	52	82	15.8	36.6
2	3	4	1	8	37.5	87.5
3	44	57	118	219	20.0	46.2
4	38	52	75	165	23.0	54.5
				Av.	24.0	56.2
Half Kernels[a]						
5	9	6	36	51	17.6	29.4
6	6	5	26	37	16.2	29.8
7	5	5	29	39	12.8	25.6
8	5	5	40	50	10.0	20.0
				Av	14.1	24.9

[a] Total aflatoxins.

accurate as the TLC procedure, but does estimate the aflatoxin level. The TLC values were obtained by the same modified procedure as mentioned above (3). To determine only whether the contamination is above or below a certain level, one standard column is sufficient. Since 30 ppb is the level of contamination generally considered to be the maximum for safety, one standard column of 30 ppb could serve as a "go/no-go" standard.

Millicolumn chromatography can also be used for detecting aflatoxin in the damaged kernels removed during peanut grading. The kernels are extracted in a Waring Blendor with 100 ml of the chloroform-methanol mixture. The column is then developed directly in the Waring Blendor container. No filtration of the extract is necessary. Warming at 60C under ultraviolet radiation for about 5 min dissipates extraneous fluorescence. Since only a few kernels are usually extracted the oil content of the extract is low and development is completed in about 5 min. The aflatoxin band is about 15 mm from the bottom of the column.

TABLE II

Quantifying MCC Method for Aflatoxin in Peanuts by Comparing Unknown Aflatoxin Columns with Standard Aflatoxin Columns of 30, 60, 100 and 150 ppb. TLC Aflatoxin Values are Given for Comparison[a]

Sample No.	MCC ppb	TLC ppb
1	100–150	119
2	<30	22
3	60–100	75
4	>150	180
5	30–60	43

[a] Total aflatoxin.

Another application of the millicolumn method is the rapid screening of extracts before spotting on TLC plates. Ten ml of the chloroform-methanol mixture are added to the dry extract and a millicolumn developed in this mixture. Columns should be warmed for 5 min at 60C under ultraviolet radiation to dissipate extraneous fluorescence. Aflatoxin is more concentrated in this application than in the other 2 applications and sensitivity is in the order of 1 or 2 ppb.

ACKNOWLEDGMENT

Most of the analytical work was done by P. C. Barnes, Physical Science Technician, Peanut Quality Investigations, Albany, Georgia.

REFERENCES

1. Lee, L. S., L. Y. Yatsu and L. A. Goldblatt, JAOCS *44*, 331–332 (1967).
2. Neshein, S., D. Barnes, L. Stoloff and A. D. Campbell, JAOCS *47*, 586 (1964).
3. Robertson, J. A. Jr., L. S. Lee, A. F. Cucullu and L. A. Goldblatt, JAOCS *42*, 467–471 (1965).

[Received April 10, 1968]