A Rapid Screening Method for the Aflatoxins and Ochratoxin A

CHARLES E. HOLADAY, National Peanut Research Laboratory,
PO Box 637, Forrester Drive, Dawson, Georgia 31742

ABSTRACT

A rapid, economical minicolumn procedure for screening aflatoxins and ochratoxin A in a wide range of products is presented. The technique includes high speed blending of the sample with aqueous methanol, purification of the extract with a solution of zinc sulfate and phosphotungstic acid, partitioning in benzene, and minicolumn chromatography. Sensitivities of 4 ppb for the aflatoxins and 20 ppb for ochratoxin A could be achieved; and the simplicity of the method, which includes the use of disposable plastic and glass items, makes the method practical for field or in-plant applications.

INTRODUCTION

Because of the seriousness of the mycotoxin problem in cereal grains and oilseeds, there has been a continuing interest in the development of a simple, rapid, and uniform screening method that would enable detection of two or more mycotoxins in a single extract after one clean-up procedure. During the past few years, several multimycotoxin detection methods were proposed (1-3). Many of these method, however, require extensive clean-up procedures and involve considerable expenditure of equipment, time, and effort. Since the successful application of the minicolumn technique to the detection and semiquantitation of the aflatoxins in many oilseed, cereal grains, and other commodities, it was believed feasible to adapt the minicolumn procedure as proposed by Holaday and Lansden (4) for the detection of both the aflatoxins and ochratoxin A after one extract and clean-up procedure. The purpose of this work was to develop such a method which would be applicable to a wide range of commodities.

The method is simple and rapid. It includes the use of plastic and glass disposable items which reduce maintenance and keep the cost per determination low. The simplicity of the method lends itself to field or in-plant use, where laboratory facilities are limited or nonexistent. The method was successfully used on wheat, barley, sorghum, corn, rice, rye, and peanuts.

EXPERIMENTAL PROCEDURE

Equipment

The equipment used is a chromatovue chamber equipped with long-wave UV, Ultra Violet Products, Inc., San Gabriel, CA; blender, Waring type; vacuum source, either a water aspirator or small vacuum pump is satisfactory. Recommended vacuum: 15-18 in. of mercury.

Minicolumns

The aflatoxin minicolumn, which has been described by Holaday and Lansden (4), is constructed from glass tubing 150 mm in length and 8 mm OD packed with ca. 15 mm of florisil (100-200 mesh) on bottom and ca. 15 mm of neutral alumina on top. The alumina should not have any background fluorescence (E. Merck and Woelm brands are recommended). The packing to hold the florisil and alumina in place can be made from either paper pulp or glass wool. The ochratoxin A minicolumn is made from the same size glass tubing as the aflatoxin minicolumn and is packed with 60 mm of florisil. Glass wool is used as packing to hold the florisil in place. (See Fig. 1). Aflatoxin minicolumns may be purchased from the Tudor Scientific Glass Co., Belvedere, SC.

Disposable Items

These include culture tubes, 20 x 150 mm; plastic tube closures, 16 mm; pipets, 1 ml; plastic funnels, 2-1/4 in. top diameter; glass fiber filter discs, 12-1/2 cm diameter; and filter paper, 15 cm diameter (S&S No. 597).

Reagents

Methanol-water extraction solvent (8/2 v/v); clean-up solution (150 g zinc sulfate, 50 g phosphotungstic acid dissolved in 1000 ml distilled water); hexane-acetone solution (8/2 v/v); benzene; methyl alcohol; 0.25 N sulfuric acid. All reagents should be ACS grade.

Procedure

For grain, pulverize the dry sample in the blender at high speed for 1 min. Add the methanol-water solution and blend for another minute. For oilseeds, blend sample with the methanol-water extraction solvent for 2 min at high speed. Any size sample may be used as long as sample weight to solvent volume is 1:2 and the capacity of the blender is not exceeded. Fold a 15 cm filter paper into a plastic funnel and filter 15 ml of the methanol-water extract into a culture tube. To it add 15 ml of the clean-up solution. Close culture tube with a plastic closure and gently mix contents by up-ending culture tube 5 or 6 times. Filter 15 ml of the mixture through a glass fiber filter disc

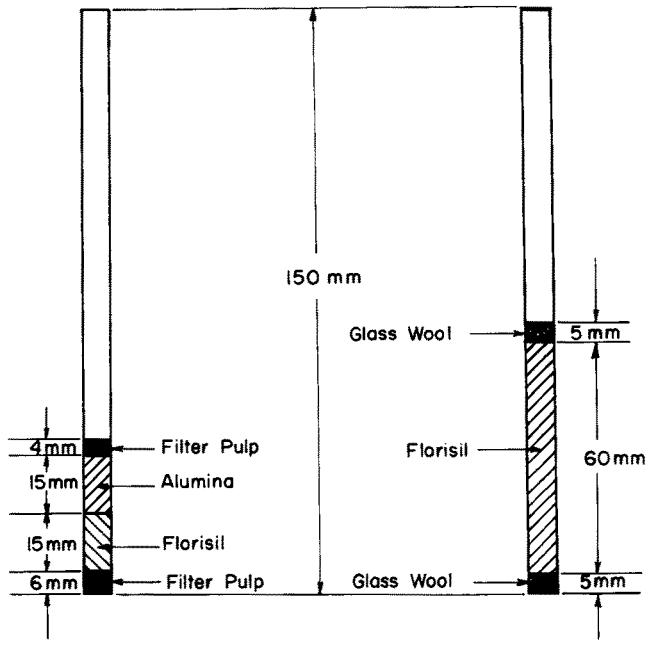

FIG. 1. Diagram of the aflatoxin (left) and the ochratoxin (right) minicolumns.

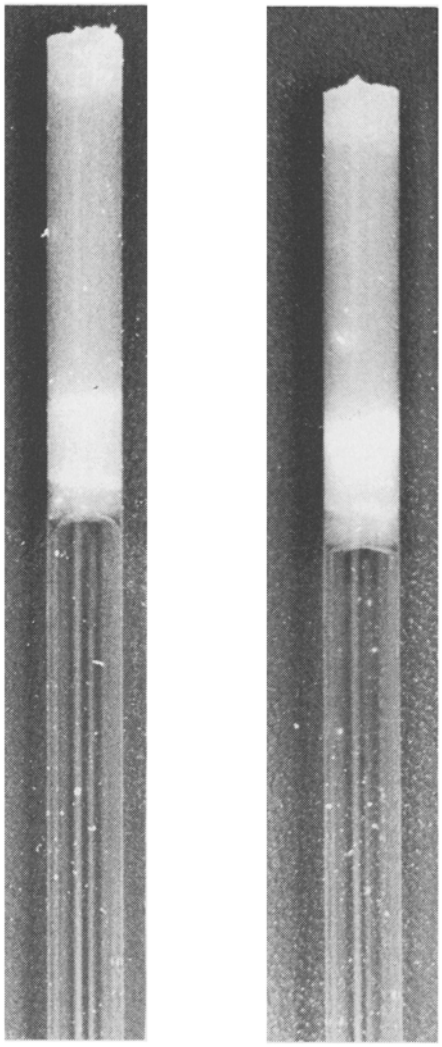

FIG. 2. Detection of ochratoxin in samples (left to right) of rice (20 ppb) and peanuts (40 ppb).

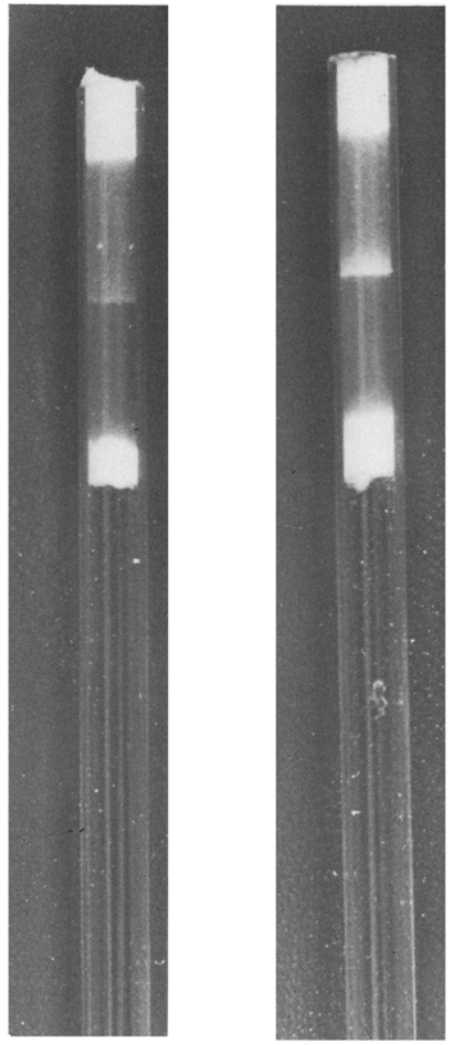

FIG. 3. Detection of aflatoxin in samples (left to right) of rice (4 ppb) and peanuts (150 ppb).

(12.5 cm), folded into the same funnel, into a second culture tube. Add 3 ml of benzene, close the culture tube with the plastic closure, and gently mix contents as before. Let layers separate and pipet 1 ml of the upper benzene layer into the top of the aflatoxin minicolumn, the bottom of which is attached to a vacuum source. After the benzene has been pulled through, add 5 ml of the hexane-acetone solution to the top of the minicolumn and pull through. Continue pulling the vacuum for an additional 2 min. Remove the minicolumn and observe under long-wave UV. A discrete, blue fluorescent band in the center of the column or at the interface of the florisil and alumina indicates at least 4 ppb of aflatoxin. To detect ochratoxin A, add 1 ml of the remaining benzene layer to an ochratoxin minicolumn, the bottom of which is attached to a vacuum source. After the benzene has been pulled through, add 3 ml of methyl alcohol to the top of the column and pull through. Continue pulling vacuum for another 15-20 sec. Remove the column from the vacuum, add 0.3 ml of 0.25 N H_2SO_4, and let it wick down the column. Immediately look at the column under a long-wave UV lamp. A discrete, blue fluorescent band ca. 1 cm from the top of the column indicates at least 8 ppb of ochratoxin A if the sample is corn, rice, or peanuts; and 16 ppb of ochratoxin A if the sample is barley, wheat, rye or sorghum. The minimum detectable fluorescence level is higher in barley, wheat, rye, and sorghum samples because of minor interferences present in these sample materials.

RESULTS AND DISCUSSION

The procedure for detecting both the aflatoxins and ochratoxin A was timed on several occasions, and averaged 15 min for completion. The most time-consuming step in the procedure was the filtration. Most extracts filtered rapidly, but some, such as those of rye, took longer so that analysis time was lengthened by a minute or so. If detection of only one of the mycotoxins were required, time for an analysis would be < 10 min.

Minute amounts of acetic acid in the benzene layer which is applied to the minicolumn would prevent retention of the ochratoxin on the florisil. Thus, the zinc acetate-acetic acid solution used for clean-up to remove interfering materials from the extract, as suggested by McKinney (5) and Holaday and Lansden (4), could not be used in this procedure since the benzene extracted minute amounts of the acetic acid from this clean-up solution. To overcome this problem, we substituted zinc sulfate for zinc acetate and phosphotungstic acid for the acetic acid. The phosphotungstic acid also improved the clean-up.

The ochratoxin minicolumns should be observed under the UV immediately after the sulfuric acid solution has wicked down the column since the ochratoxin band starts spreading within about 1 min. Occasionally, a faint, white fluorescent band appeared when samples of barley, rye, sorghum, and wheat were analyzed, but gave no problem. For best results, the aflatoxin column should be viewed

with only overhead UV light; the ochratoxin column should be viewed with UV lights placed overhead and beneath it.

The procedure was checked by analyzing samples of corn, wheat, rye, barley, sorghum, rice, and peanuts spiked with ochratoxin A and the four aflatoxins. The ochratoxin levels ranged from 8 ppb to 250 ppb, and the aflatoxins ranged from 4 ppb to 150 ppb. The samples had been previously analyzed and found to be free of both mycotoxins. Figure 2 illustrates the sensitivity of this procedure for ochratoxin A. Figure 3 illustrates the sensitivity of this procedure for the four aflatoxins ($B_1+B_2+G_1+G_2$). Although ochratoxin A and the aflatoxins have not been found naturally occurring together on the same sample, all commodities mentioned in this report have at one time been found to be naturally contaminated by either one or the other of the mycotoxins. For experimental purposes, in order to demonstrate the utility of the procedure, these samples were spiked with both ochratoxin A and the four aflatoxins. Ten samples of corn, wheat, and sorghum, naturally contaminated with ochratoxin A ranging in levels from 20 to 110 ppb in the corn, 50 to 250 ppb in the wheat, and 75 to 300 ppb in the sorghum, were tested by both the Scott (3) and minicolumn methods, and, in every case, the minicolumn method showed positive for those samples containing ochratoxin A. One hundred samples of corn and peanuts naturally contaminated with all four aflatoxins, ranging in levels from 15 ppb to 50 ppb in the corn and from 10 ppb to 125 ppb in the peanuts, were tested by both the AOAC official (6) and minicolumn methods. Without exception, samples shown to be contaminated by the official method also tested positive on the minicolumn.

The minicolumn method should be useful for screening, to indicate "go" or "no-go." For example, 0.5 ml of benzene extract from a sample prepared in accordance with the method outlined in Experimental Procedures would give a positive response for aflatoxin at 8 ppb and a positive response for ochratoxin at 16 ppb. Absence of blue fluorescent band on either minicolumn would indicate < 8 ppb of aflatoxin and < 16 ppb of ochratoxin A.

The minicolumn method could also be used for semiquantitation by the addition of small increments of the extract to the minicolumn until a band became visible. Thus, if no band were visible after addition of 0.25 ml but one were visible after addition of 0.5 ml, the level of aflatoxin would be between 8 and 16 ppb; similar results for ochratoxin A would indicate between 16 and 32 ppb (corn). These are only estimates, of course, but give an approximation of the aflatoxin and ochratoxin A levels.

Reference aflatoxin and ochratoxin solutions may be prepared in benzene. The solutions would be applied to the minicolumns in the same manner as sample extract, and the minicolumns could then be used as reference columns. Unskilled people could be trained to use them to semiquantitate the mycotoxins on other columns. The aflatoxins are unstable in UV light, so fresh columns should be prepared daily. Ochratoxin A is more stable but will, in time, decompose upon repeated exposure to UV. Also, as mentioned, the ochratoxin band will spread after about 1 min, so new standard columns would have to be made for each test.

REFERENCES

1. Eppley, R.M., J. Assoc. Off. Anal. Chem. 51:74 (1968).
2. Stoloff, L., Stanley Nesheim, Lillian Yin, J.V. Rodricks, Michael Stack, and J.D. Campbell, Ibid. 54:91 (1971).
3. Scott, P.M., and T.B. Hand, Ibid. 50:366 (1967).
4. Holaday, C.E., and John Lansden, J. Agric. Food Chem. 23:1134 (1975).
5. McKinney, J.D., J. Assoc. Off. Anal. Chem. 42:213 (1975).
6. "Official Methods of Analysis," 12th edition, Association of Official Analytical Chemists, Washington, DC, 1975, Methods 26.014-26.019.

[Received April 8, 1976]