

Detection of Aflatoxin Using Small Columns of Florisil

ABSTRACT

This simplified procedure to detect aflatoxin in cottonseed and cottonseed products can be used at the field level where equipment for thin layer chromatography is not available. Contamination levels as low as 10 ppb can be detected in these columns or tubes using longwave uv light. Development and detection of aflatoxin in these tubes takes ca. 10 min.

The cottonseed industry is still seeking means of decreasing the time required to determine whether a seed lot or a batch of meal is contaminated with aflatoxin. The economics involved in products contaminated with aflatoxin are quite apparent. For this reason oilseed laboratories have continued their investigations of simplifying aflatoxin methodology.

We have developed a small column or tube layered with adsorbents of varying activities (Fig. 1) to replace the resolution of aflatoxin on thin layer plates. Holaday (1) suggested the use of small silica gel columns to detect aflatoxin in peanut products; however these columns were not adequate for cottonseed products because of the large amounts of gossypol pigments.

Preliminary purification of extracts is necessary to

remove the major portion of these interfering pigments. Extract solutions are purified with ferric gel, and residues equivalent to 5 g are added to the tubes in 2 ml of chloroform-acetone 9:1 solution. As the solution passes through the column remaining pigment impurities are adsorbed on the top silica gel layer while the aflatoxin fraction passes through and is adsorbed by the more active florisil layer.

The entire procedure can be completed in ca. 45 min if sample is extracted in blender.

Materials required are: (a) pyrex glass tubing, 4 mm ID, cut to 9-10 in. lengths; (b) glass wool; (c) sand; (d) florisil, 100-200 mesh; (e) silica gel for column chromatography, E. Merck or equivalent; (f) alumina, acid, 80-200 mesh,

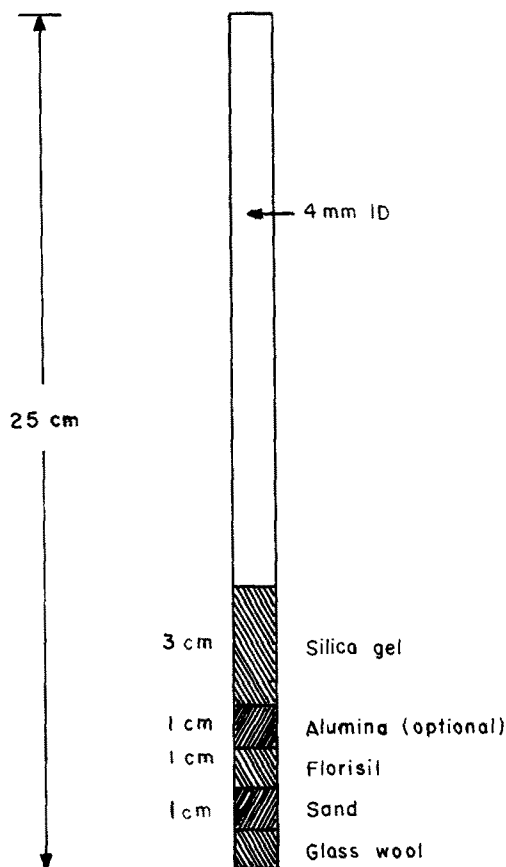


FIG. 1. Schematic diagram of florisil tube.

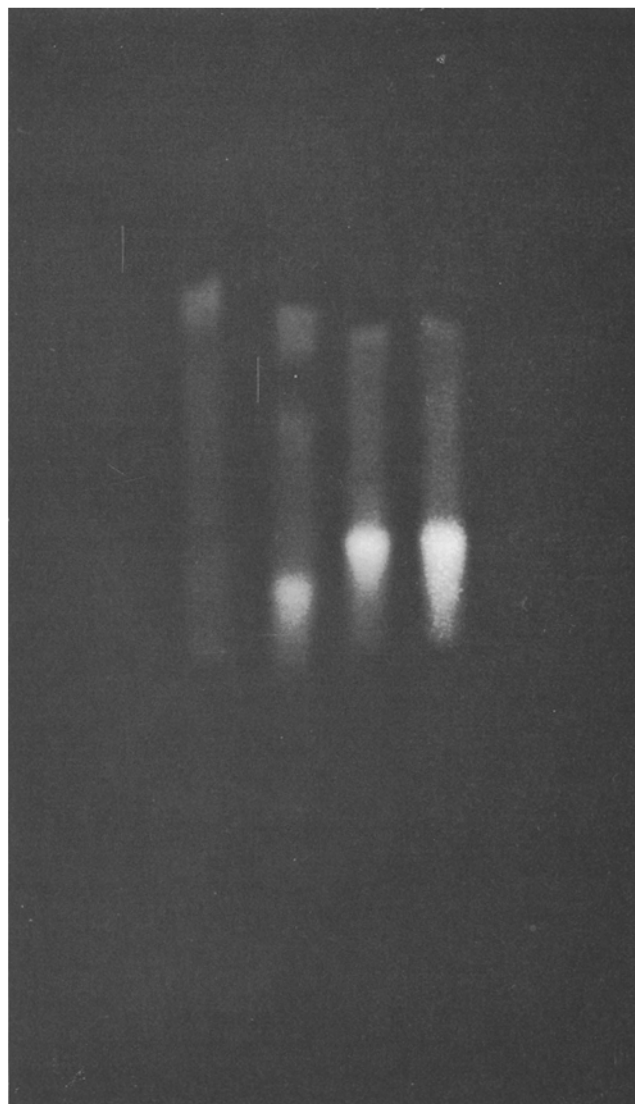


FIG. 2. Detection of aflatoxin in field samples of cottonseed and cottonseed meals. Levels of contamination, from left to right, are equivalent to 0, 8, 19 and 53 ppb or $\mu\text{g}/\text{kg}$.

Brockman Activity I; (g) longwave uv light, one 15-watt tube 18 in. long.

Adsorbents are activated at 110 C for 2 hr. A small funnel and scoops to deliver 1 and 3 cm layers are used to transfer the dry adsorbents into the tubes. Because the fluorescent intensity in the florisil layer decreases with increased diameter and length, these dimensions are kept to a minimum. The cost of materials per tube is less than 1 cent and the time required to prepare a tube is less than 1 min. The glass tubes may be reused.

Extracts of cottonseed or cottonseed meal are purified by the ferric gel procedure (2). The purified extract residue (equivalent to 10 g) is dissolved and transferred into a vial with two 2 ml portions of chloroform-acetone 9:1 and mixed well by swirling. A 2 ml aliquot (equivalent to 5 g) is transferred with a pipet into the tube and allowed to drain. The tube is refilled with chloroform from a squeeze bottle and about one-half the volume is allowed to drain. The tube is removed from the holder and the remaining solvent is decanted and discarded. The drain time for 2 ml is ca. 5 min.

The tube is placed in a viewing cabinet and examined under uv light. A definite fluorescent glow will appear in the florisil layer if the aflatoxin content is above 50 ppb (1 ppb = 1 ng/g). A 5 g equivalent of seed or meal containing 50 ng of aflatoxin would be equivalent to 10 ng/g or 10 ppb.

The amount of pigment impurities will vary with samples. Meals generally contain more impurities than seed. In some cases it is necessary to increase the adsorption capacity for impurities by the addition of a layer of acid alumina. At other times the amount of impurities can be decreased by reducing the amount of residue added to the

tubes to a 2-3 g equivalent.

Background interference in the silica gel layer is minimized by adjusting the intensity of the uv light. We obtain good resolution by placing the florisil tubes ca. 4 in. from a single 18 in. uv lamp.

Figure 2 shows the detection of aflatoxin in field samples of cottonseed (B,D) and cottonseed meals (A,C) using tubes layered with silica gel and florisil. Varying levels of contamination in 5 g equivalents are shown. The sample on the left (A) shows no detectable amount of aflatoxin, while samples B, C and D show 39, 97 and 264 ng, respectively, as determined by thin layer chromatography. This would be equivalent to 0, 8, 19 and 53 ppb or $\mu\text{g}/\text{kg}$. Unknown contaminations can be estimated by comparison with standard tubes at the critical aflatoxin levels. An equivalent of noncontaminated residue must be added to the standard aflatoxin in order to provide normal background interference found in the residues.

A critical evaluation of the sensitivity and variance of detection of aflatoxin in these tubes is currently underway and will be reported.

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REFERENCES

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2. Velasco, J., J. Ass. Offic. Anal. Chem. 53:511 (1970).

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