Minicolumn Screening Methods for Detecting Aflatoxin: State of the Art

T.R. ROMER, N. GHOURI, and T.M. BOLING, Ralston Purina Company,
Checkerboard Square, St. Louis, MO 63188

ABSTRACT

The use of small chromatographic columns (minicolumns) for the detection of aflatoxin in food or feed extracts was introduced in 1968. Since then many different analytical methods for aflatoxin which involve a minicolumn detection step have been developed. Four of these have been adopted as official Association of Official Analytical Chemists (AOAC) procedures. The advantages and disadvantages of the types of minicolumns along with a comparison of the minicolumn technique to thin layer chromatography is discussed.

INTRODUCTION

Holaday (1) introduced the minicolumn technique in 1968. In this, the first analytical method of this kind, the minicolumn was used to detect aflatoxin in peanuts in a manner similar to that in which a thin layer chromatography (TLC) plate is traditionally used. The minicolumn consisted of a section of 4 mm (ID) glass tubing ca. 75 mm in length, containing: (a) a 5.0 mm glass fiber plug to hold the packing material in place; (b) 45 mm of silica gel; and (c) another 5.0 mm glass fiber plug. The minicolumn was placed in a beaker containing a "developing solvent" which was drawn up the column by capillary action. After 10-15 min the column was removed from the beaker and examined under longwave ultraviolet (UV) light for the characteristic blue or bluish-green color that the aflatoxins emit when exicted by light of this (365 nm) wavelength. The two main advantages of this first minicolumn technique for detecting aflatoxins in peanuts over the TLC methods available at that time (2-4) were that the minicolumn method was both more rapid (25 min vs. 2 hr) and simpler to use. In order to distinguish between this first type of minicolumn and those that were developed later, we shall call this first type of minicolumn, because it was "dipped" into a solution containing aflatoxin, the Holaday "dip" column.

Modification of the Holaday "Dip" Column

In 1972 Cucullu et al. (5) reported a screening method for the detection of aflatoxin in cottonseed products which used a slightly modified Holaday "dip" column. The modification involved the use of a small layer (15 mm) of acidic alumina beneath 90 mm of silica gel. Thus, in the development of this "dip" column, the sample extract would first pass through the alumina and then through the silica gel. The alumina would serve the purpose of removing certain pigments, etc., from the extract that would otherwise interfere with the detection of aflatoxins on the silica gel. This method was modified by Pons et al. (6) and then by Shannon et al. (7) until finally it became an official Association of Official Analytical Chemists (AOAC) screening method for aflatoxin in corn (8).

Limitations of the "Dip" Column

Although the use of a minicolumn was, as stated previously, more rapid and simpler than the available TLC methods, the "dip" column technique suffered from some serious limitations. These limitations were (a) the volume of sample extract that was drawn up the column packing varied from column to column; (b) the final height of the aflatoxin band varied from column to column; and (c) the aflatoxin band would spread soon after the column was removed from the sample extract. The first limitation impaired the quantitative aspect of this technique. The second limitation seriously hindered the qualitative accuracy of this technique, and the third limitation imposed a variability onto the sensitivity of the method, since the diffusion or spreading caused the fluorescent aflatoxin band to become dimmer with time.

Improvement on the "Dip" Column

In the same year (1972) that Cucullu (5) reported the use of alumina as a second adsorbent in the "dip" column of Holaday, Velasco (9) reported the optional use of alumina in a minicolumn in a method for detecting aflatoxin in cottonseed products, but in this case the minicolumn was not a "dip" column, but rather was one to which a specified volume of sample extract added to the top of the column was allowed to drain through the packing material under the force of gravity. By incororating into a minicolumn method the use of a constant volume of sample extract, Velasco had overcome the first "dip" column limitation listed above. The other two limitations were also overcome by Velasco in the novel use of florisil beneath the silica gel in a minicolumn. With the elution solvent used (9:1, chloroform/acetone), aflatoxin will attach itself to the top of the florisil layer in a tight band. With the use of florisil in this manner, there is no doubt where the aflatoxin from standard solution or sample extract will be banded on the minicolumn, nor is there any problem with diffusion of the aflatoxin band after the band has been formed. Velasco also incorporated the use of a layer of sand immediately beneath the florisil to provide an even base for the florisil. Later, when Velasco developed a screening method for aflatoxin in corn which became an official AOAC method (10), a layer of alumina on top of the silica gel was incorporated as a permanent part of the minicolumn.

Other Methods That Use the Velasco Minicolumn

In 1975 Romer (11) modified the Velasco minicolumn and incorporated the modified column into a method for detecting aflatoxin in 24 agricultural commodities. This method has since become an official method for the AOAC (12). The two modifications that Romer incorporated into the Velasco minicolumn were: (a) calcium sulfate was added to both ends of the minicolumn, replacing the sand on the lower end, and (b) the I.D. of the glass tubing was changed from 3 mm to 6 mm. The calcium sulfate at the top of the minicolumn removes any residual water from a solution added to the minicolumn; the calcium sulfate at the bottom provides an even base for the florisil and also, because of the relatively large granules used (20-40 mesh), allows for a more rapid flow of solution through the column than would the florisil if it were the bottom layer. Both the top and bottom layers of calcium sulfate keep the inner layers (florisil, silica gel and alumina) of the minicolumn packing free of moisture for a short period of time after the column is removed from a desiccator. Moisture on any or all of the three inner layers can render the mini-

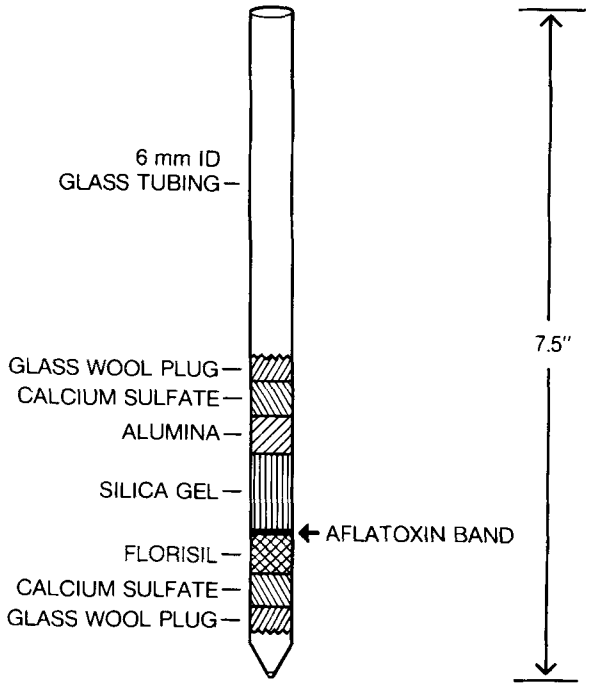

FIG. 1. Velasco minicolumn.

column nonfunctional. The use of larger diameter glass tubing (6 mm vs. 3 mm I.D.) provides a minicolumn that is much easier to work with and can hold 3 ml of solvent on top of the packing without becoming so long that it is unwieldy. Figure 1 shows a sketch of this version of the Velasco column. This is the type of Velasco column that is commercially available (13).

In 1974 Barabolak (14) reported a method for aflatoxin in corn products which used a Velasco minicolumn. This method has since been shortened, and the shortened version has been successfully tested in an AOAC collaborative study (O. Shotwell, personal communication, 1978, results to appear in future issue of JAOAC). Along with the modified Barabolak method, a method reported by Holaday in 1975 (15) was tested in this same collaborative study (O. Shotwell, personal communication, 1978, results to appear in future issue of JAOAC). The latter method uses another modification of the Velasco minicolumn which is referred to as the Holaday minicolumn. In this version, the activated (dry) silica gel and alumina, which had been packed on top of the florisil are replaced by alumnina that contains ca. 15% water. This column is also commercially available. (MycoLab Co. provides two types of Holaday columns, one that requires a vacuum source and one that does not. Ag. Science Corp., PO Box 253, Shellman, GA, 31786, and Tudor Scientific Glass Co., 555 Edgefield Rd., Belvedere, SC, 29841 also provide Holaday columns.) In the collaborative study, both the Velasco column and the Holaday column were tested as part of Holaday's method. When the Velasco column was used, the elution solvent that was published in the original method (9:1, $CHCl_3$/Acetone) (7) was used. The results of this collaborative study show that, although both the Velasco and Holaday minicolumns can be used with Holaday's method, the Velasco minicolumn gives better results. Holaday's 1975 method with the Velasco minicolumn is now an official AOAC method.

The Use of Vacuum with Minicolumn

Any of the methods that use a Velasco column or a modification of it can be completed in less time if a small vacuum is used to drain the sample extract and elution solvent through the column. In fact, since the Holaday columns are packed using paper pulp on both ends to hold the adsorbents in place, a vacuum is essential to complete the method in less than an hour. However, if too strong a vacuum is used, the solutions placed on the minicolumn will travel through the adsorbents fast enough to cause interferences and/or aflatoxin to spread over the adsorbents. As the aflatoxin spreads over more of the florisil layer, the bluish fluorescence becomes dimmer and dimmer until finally a band, which would be very intense if no vacuum were used, will not be detectable. If interferences on the alumnina or silica gel layers spread sufficiently to reach the florisil layer, they will cover any aflatoxin present. This, also, tends to dim the fluorescent intensity of the aflatoxin band. A vacuum should be used that drains a solution through the minicolumn no faster than 1.0 ml/min to ensure that spreading of the aflatoxin or interferring bands will not take place.

Major Uses of Minicolumn Tests

The two major uses of minicolumn tests for aflatoxin are: (a) as "go or no go" field tests to accept or reject a truckload or railroad car of peanuts or corn, and (b) as central laboratory screening tests to reduce the time necessary to test samples that do not contain a detectable amount of aflatoxin. The main reasons that minicolumn tests are so widely used at the plant or field level to accept or reject lots of peanuts and corn are the same reasons that the minicolumn test is preferred to TLC at the plant level; i.e., little time or expertise is required. The minicolumn method that is popular at the central laboratory level (11,12), where the elapsed time is often not as important as at the plant level, retains this popularity because of these characteristics: (a) it applies to virtually all commodities that one might wish to test for aflatoxin; (b) if aflatoxin is detected in a sample, some of the same solution that is used for the minicolumn test can be used for confirmation and quantitation of the aflatoxin present; and (c) this method is an official method of the Association of Official Analytical Chemists (AOAC), the American Association of Cereal Chemistry (AACC), and the International Union of Pure and Applied Chemists (IUPAC).

SOLVENTS

Some of the minicolumn methods use solvents that are known carcinogens, such as benzene and chloroform. In most methods, toluene can be substituted for benzene, and methylene chloride for chloroform. However, controlled experiments should be performed to demonstrate that the method performs well with the solvent substitute.

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