Rapid Screening Method for Zearalenone in Corn, Wheat and Sorghum

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ABSTRACT

A rapid and sensitive screening method has been developed for zearalenone in corn, wheat and sorghum. The method is based on minicolumn chromatography, and an analysis can be completed in ca. 10 min in most cases. Because several items used are disposable, the amount of cleanup is reduced and the cost per determination is maintained at a low level.

INTRODUCTION

Zearalenone, or F-2 toxin, is produced by various species of *Fusarium*, particularly by *F. roseum* Graminearum *(Gibberella zeae)* (1). The toxin has been found in the seed of wheat, oats, barley and especially corn (2,3). Recent reviews have described the chemistry and estrogenic effects of zearalenone and its occurrence in foods and feeds (4-7).

Methods of analyses include thin layer, gas liquid and high pressure liquid chromatographic procedures (8-18). None of these procedures, however, lends itself to a low-cost screening method where laboratory facilities are limited or nonexistent. While conducting research on zearalenone over the past 2 years, we found that its fluorescence under long-wave UV lighting, when spotted on alumina, was several times greater than when spotted on silica gel; its fluorescence under short-wave UV lighting was not as intense as under longwave UV lighting when spotted on alumina. We do not claim this finding to be original, although we have not seen it in the literature. Through this finding, we felt we could develop a rapid screening method for zearalenone, as well as a thin layer chromatographic (TLC) procedure that would be more sensitive than current TLC methods.

This paper describes a rapid screening method for zearalenone, which has a sensitivity of ca. 35 ppb. It is based on the minicolumn technique and can be used at field locations. Disposable items were used to reduce the amount of maintenance and glassware cleanup, keep cost per determination low and decrease the possibility of zearalenone carryover to a subsequent sample.

MATERIALS AND METHODS

Equipment

(1) Chromatovue chamber equipped with long-wave UV lighting: Ultra-Violet Products, Inc., San Gabriel, CA;
(2) blender—Waring, or equivalent, explosion-proof;
(3) vacuum source—small vacuum pump or water aspirator;
(4) test tube rack to accomodate tubes 20 mm in diameter;
(5) tilt-type pipets—one 3 ml capacity and one 15 ml capacity;
(6) rubber pipet fillers;
(7) one or more 1000-ml filtering flasks, depending on how many samples will be analyzed at the same time;
(8) a size 8, one-hole rubber stopper for each filtering flask;
(9) plastic T's if more than one filtering flask is used;
(10) 1.5-meter length of rubber vacuum hose to connect filtering flask to vacuum source;
(11) pipet, 5 ml capacity.

Minicolumns

Glass tubing 200 mm in length and 5.5 ID was packed with ca. 1.5 cm of neutral alumina (70-230 mesh) on the bottom and Florisil (5 cm 100-200 mesh) on top. The alumina was prepared by adding 8% (wet basis) distilled water to activity level I alumina. After the water was added, the alumina was thoroughly mixed and all lumps were broken up while inside a closed container. This prevents moisture loss when the material heats up during the addition of H_2O. The adsorbent was equilibrated in an airtight container for 24 hr before it was used to prepare the columns. The Florisil was prepared by adding 12% distilled H_2O (wet basis). We have found that some lots of Florisil may contain as much as 1-3% moisture when received. It is recommended, therefore, that a moisture determination be made on the Florisil before the water is added to avoid adding too much. This is accomplished by weighing 10-15 g of the material into aluminum boxes and placing the moisture boxes in a forced-draft oven operating at 175 C for 12 hr. The boxes should be cooled in a desiccator when removed from the oven. After the water was added, the Florisil was mixed and allowed to equilibrate in similar fashion to the alumina. The interface of the alumina and the Florisil should be as straight as possible. Packing to hold the Florisil and alumina in place was paper pulp (Schleicher & Schuell No. 289) available from most chemical supply houses (see Figure 1). The minicolumns should be stored in an airtight container, not in a desiccator. Before minicolumns are used, they should be tapped and the upper packing pressed down against the Florisil to make sure the adsorbents are firmly packed.

Disposable Items

(1) Culture tubes—20 x 150 mm;
(2) plastic stoppers—hollow, 20 mm bottom diam. (Mallinckrodt P310, obtainable from Curtin Matheson Scientific, Inc.);
(3) pipets (glass)—1 ml;
(4) plastic funnels—5.7 cm top diam.;
(5) filter paper—15 cm, S&S No. 597;
(6) glass fiber filters, Reeve Angel No. 934 AH, 12.5 cm diam.

Reagents

(1) Toluene;
(2) methanol/water solution (80:20, v/v);
(3) salt solution (distilled water with 15% sodium chloride, 15% zinc acetate and 0.375% glacial acetic acid or 4 ml/1000 ml water);
(4) hexane/acetone solution (90:10, v/v);

All reagents should be ACS grade.

Procedure

We ground a sample in the blender for 1 min, then blended it with the methanol/water solution (2 ml/g sample) for an additional min at high speed. A 100-g sample

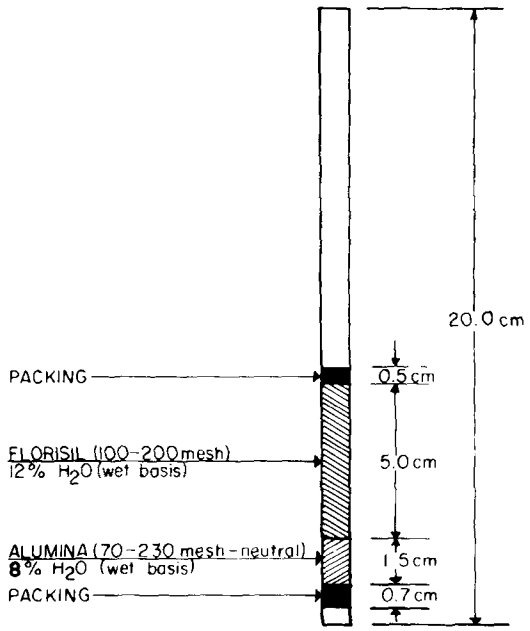

FIG. 1. Zearalenone minicolumn.

properly prepared by subsampling from a representative, thoroughly blended primary sample should be used. Fifteen ml of the extract was filtered into a culture tube using the 15-cm filter paper. To this extract, we added 15 ml of the salt solution. The culture tube was closed and shaken vigorously for a few sec. Fifteen ml of this solution was filtered into a second culture tube using the 12.5-cm glass fiber filter and then 3 ml of toluene was added. The tube was shaken very gently for a few sec and the layers were allowed to separate. One ml of the toluene layer (upper layer) was then pipetted into the top of the minicolumn, the lower end of which was attached to a vacuum source. After the toluene was drawn down into the minicolumn, we added 5 ml of the eluting solution (hexane/acetone) to the top of the minicolumn and pulled it through. The vacuum was adjusted such that the 5 ml would be pulled through in ca. 60 sec. We removed the minicolumn and placed it under a long-wave UV light source. A blue band at the interface of the Florisil and the alumina indicated at least 35 ppb of zearalenone. The sensitivity was determined by spiking a zearalenone-free corn sample with a zearalenone standard solution (available from Myco-Lab Company) in an amount equivalent to 35 ppb. The addition of 0.2 ml instead of 1.0 ml of the toluene layer to the minicolumn reduced the sensitivity to ca. 175 ppb of zearalenone. If the concentration of zearalenone in the sample is 1000 ppb or more, which is often the case, a dilution technique may be used to determine the approximate level. This procedure is accomplished by diluting 1 ml of the toluene layer and adding small increments of this solution to the minicolumns until a band is visible. The approximate zearalenone level may be determined by taking into account the dilution and the number and size of the increments added to the minicolumn.

RESULTS AND DISCUSSION

The minicolumn developed for zearalenone is shown in Figure 1. Extensive testing has shown that this design works best for most applications. Other designs in which we used either acid or basic alumina and 60-100 mesh Florisil did not work as well. Time for a test is ca. 10 min for corn and

TABLE I

Comparison of Thin Layer Chromatography and Minicolumn Analyses of Zearalenone in Corn, Wheat and Grain Sorghum

Sample	PPB-minicolumn	PPB-TLC
1 - corn	3500 - 4600	4400
2 - corn	2800 - 3500	3000
3 - corn	233 - 350	250
4 - corn	1400 - 1520	1600
5 - corn	175 - 350	300
1 - wheat	5830 - 6360	7500
2 - wheat	5830 - 6360	7500
3 - wheat	820 - 875	1030
4 - wheat	<35 -	<200
5 - wheat	435 - 500	615
1 - grain sorghum	35 - 45	<200
2 - grain sorghum	5830 - 6360	6950
3 - grain sorghum	585 - 700	878
4 - grain sorghum	1000 - 1165	1450
5 - grain sorghum	585 - 700	788

sorghum; however, wheat causes some minor filtering problems. A wheat sample can also cause problems in the toluene partition step because of an emulsion formation unless the toluene is mixed very gently with the other contents in the culture tube. Table I shows a few of the many tests run on naturally contaminated samples by both the minicolumn and TLC. The zearalenone values from the minicolumns were estimated by diluting 1 ml of the toluene layer by as much as 200-to-1 and adding increments of 0.1-0.2 ml to the minicolumn until a band became visible. The TLC analyses on the wheat and sorghum have already been performed by Shotwell (2); the levels of zearalenone may have decreased with time. The sensitivity of the TLC method she used was 200 ppb. The TLC analyses on the corn samples were carried out in this laboratory according to the Scott et al. method (16). The corn samples were obtained from the Midwest, the Northeastern States and the mid-Atlantic States.

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