Fluorometric Measurement of Aflatoxins[1]

Abstract

An accurate, precise and sensitive fluorometric method for quantifying purified aflatoxins in solution is proposed. After purification, aflatoxin samples are read in a fluorometer with a primary filter of 365 nm and a secondary filter of 435 nm for B_1 and B_2 and 465 nm for G_1 and G_2. The method is precise at as little as 0.0002 μg/ml B_1 and G_1 and 0.00006 μg/ml B_2 and G_2, and accurate at concentrations of 0.002 μg/ml B_1 and G_1 and 0.0006 μg/ml B_2 and G_2.

Fluorometric Measurement of Aflatoxins

Visual estimation and fluorodensitometric quantification are used to measure purified aflatoxin extracts. In assaying large numbers of samples, visual estimation appears to be most useful, whereas fluorodensitometry is practical for a more exact evaluation of a smaller number of samples (1). Visual measurements of aflatoxin have a precision range of ±20–28% under ideal conditions (2), and when associated with the errors inherent in the entire analytical procedure approaches ±25–50% (2–5). Fluorodensitometry is more accurate than are visual techniques, with the precision range ideally varying from ±2–4% (6), and about ±5% for scans of purified toxin chromatographs (7). Fluorodensitometry of thin layer chromatography (TLC) plates does have the disadvantage of requiring removal of aliquots of toxin from solution for measurement.

The purpose of this work was to develop a technique with an accuracy of ±5% and a precision of evaluation such that the standard deviation of repeated measurements of the same sample divided by the mean of these measurements does not exceed ±0.05, a sensitivity of at least 0.2 μg/ml of aflatoxin, and a capability of continuously monitoring solutions without removing aliquots for TLC resolution.

The procedure suggested here involves the use of a fluorometer. A G. K. Turner model 300 with accompanying cuvettes were utilized in this work. If the toxin source is agriculture products, it is necessary to extract and rigorously purify the toxins. That procedure has been adequately reviewed by Pons and Goldblatt (5). Chromatographic or spectroscopic grade methanol is used as a solvent for aflatoxin as both chloroform and acetone may have high and variable background fluorescence. The primary filter used in the fluorometer is 365 nm; the secondary filter for B_1 and B_2 is 435 nm, and for G_1 and G_2 465 nm. Figure 1 shows a standard curve for aflatoxins B_1, B_2, G_1 and G_2.

This method is accurate, precise and sensitive. If exact measurements are to be made of low concentrations of toxin, care must be taken to correctly calibrate the instrument and remove all dust particles from the cuvette. In Table I, the accuracy for a sample containing 0.02 μg/ml of B_1 or G_1 and 0.006 μg/ml B_2 or G_2 ranges from ±1.3–5.0%. At dilutions as high as 0.002 μg/ml B_1 and G_1, and 0.0006 μg/ml B_2 and G_2 accuracy from the average of three readings is approximately the same as that expected from fluorodensitometry. From Table II, the precision, as measured by $\sigma/\bar{x}$, is less than 0.1 in all but one case. This means that as little as 0.0002 μg/ml B_1 and G_1 and 0.00006 μg/ml B_2 and G_2 can be detected with percision but not necessarily with total accuracy.

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TABLE I

Accuracy of Aflatoxin Determination Reported as ±% Variation

Amount toxin	B_1	B_2	G_1	G_2
0.1 X[a]	5.0	1.3	2.0	4.33
0.01 X	10.0	16.6	3.0	0.00
0.001 X	60.0	33.0	50.0	66.00

[a] X is 0.2 μg/ml for B_1 and G_1, and 0.06 μg/ml for B_2 and G_2 in methanol. These samples were used for calculation of the fluorescence constant.

TABLE II

Precision of Fluorometric Determination of Aflatoxin Reported as $\sigma/\bar{x}$[a]

Amount toxin	B_1	B_2	G_1	G_2
X[b]	0.02	0.06	0.04	0.02
0.1 X	0.01	0.05	0.19	0.04
0.01 X	0.02	0.03	0.03	0.008
0.001 X	0.02	0.02	0.06	0.07

[a] Where σ is the standard deviation with $N = 3$, and $\bar{x}$ is the mean. [b] Where X is 0.2 μg/ml for B_1 and G_1 and 0.06 μg/ml for B_2 and G_2 in 5 ml methanol.

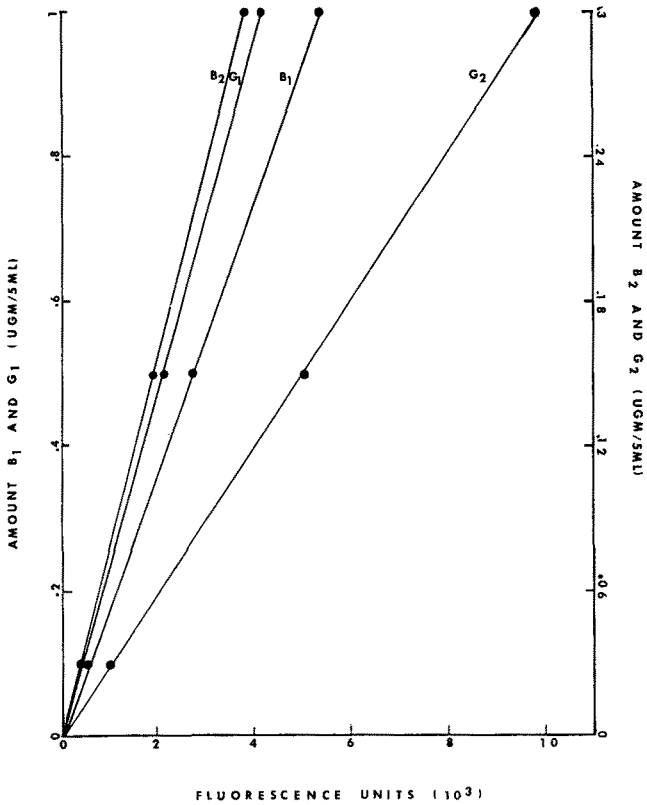

FIG. 1. Standard fluorescence curve for aflatoxins in methanol solution. Primary filter is 365 nm. Secondary filter is 435 nm for B_1 and B_2, and 465 nm for G_1 and G_2.

The fluorometric method appears to be most useful when the amount of aflatoxin in solution is to be constantly monitored (e.g., assaying chromatographic column effluents), when there is a desire to demonstrate less than 0.002 μg/ml B_1 or G_1 and 0.0006 μg/ml B_2 and G_2 in a sample solution, and when there is a need to retain the solution during quantification of toxin.

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