tract, the first wash being 50% water in methanol, the second plain water; (*6*) use silica gel column cleanup step (see *Results and Discussion*), eluting aflatoxin M with 200 ml 3% methanol in chloroform and discarding the first 50 ml; (*7*) perform TLC, using silica gel:$CaSO_4$ (1:1, w/w) adsorbent mixture and 5% methanol in chloroform as developing solvent; (*8*) quantitate spectrophotometrically (by measuring absorbance at 357 mμ of the eluate of the scraped band of aflatoxin M) or by means of visual or densitometric comparison under ultraviolet light.

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Survey of Green Coffee for Potential Aflatoxin Contamination

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A method for the detection and estimation of aflatoxin B_1 in green coffee beans is described. According to the AOAC method, the toxin is extracted with aqueous methanol: hexane and the extract is cleaned up on a Celite column. The extract is further purified on a Florisil column and is then chromatographed on thin layer plates. The estimated limit of detection of the method is 2 μg aflatoxin/kg green coffee. About 75% aflatoxin B_1 added to green coffee was recovered. The method was used in a survey of suspect samples of green coffee. No aflatoxin was detected in 58 samples originating in 10 producing countries. Preliminary studies with a new extraction solvent (dimethyl sulfoxide:benzene, 30:50) and a Florisil cleanup appear promising. About 85% aflatoxin B_1 added to green coffee was recovered.

Agricultural commodities obtained from tropical areas are likely to be contaminated with aflatoxin because the primitive farming practices are conducive to mold infection and proliferation (1). Since coffee and cocoa are commodities of significant economic value, a survey was planned to assess the incidence of aflatoxin in these materials. For screening purposes, aflatoxin B_1 was adopted as an index of contamination. To our knowledge, it

is always present in naturally contaminated agricultural commodities. Before examining samples, we had to develop an analytical method for these caffeine-containing materials. Caffeine interfered in the methods commonly used at the time this study was initiated in 1964. When the extract obtained by the AOAC procedure (2) was further purified on a Florisil column (3), the chromatograms could be easily interpreted.

The extraction step was investigated by direct addition of known amounts of toxin to the beans. Caffeine is intimately distributed within the bean cell while aflatoxin, resulting from topical growth of the producing mold, is probably not as intimately distributed nor as difficult to extract. Since aflatoxin B_1 and caffeine have such similar solubility characteristics in the solvents used, both compounds should be extracted; caffeine could be completely extracted with the solvents used. Analysis of green coffee beans with various degrees of mold attack gave satisfactory results with added aflatoxin B_1.

METHOD

Sample Preparation

Freeze the coffee beans with Dry Ice or liquid nitrogen and then grind them to a fine powder in a high speed blender. Let the freezing medium evaporate before weighing the sample.

Extraction

Extract the aflatoxin from a 50 g sample, using the official, first action method for peanuts and peanut products (extraction in aqueous methanol:hexane followed by cleanup in Celite column) (2). Dry the resulting chloroform:hexane extract eluted from the Celite column on a steam bath, under a stream of nitrogen, and add to a column packed with 9 g Florisil prepared by the procedure of Heusinkveld *et al.* (3); use about 50 ml tetrahydrofuran to dissolve and transfer the residue to the column and elute with 250 ml tetrahydrofuran. Discard this eluate. Elute the aflatoxin with 500 ml acetone. Dry the acetone eluate on a steam bath under a stream of nitrogen. Transfer to a 4 dram vial and evaporate the solvent. The residue represents material extracted from 10 g coffee. Refrigerate and avoid unnecessary exposure to light until ready to chromatograph.

Thin Layer Chromatography

Add 500 μl chloroform to vials containing the sample extract. Use 20 × 20 cm TLC plates coated with silica gel G-HR (about 0.3 cm layer), activated 2 hr at 80°C and stored in a desiccator.

Spot 25 μl sample and 5 μl standard aflatoxin B_1 solution (1 μg/ml) on the same plate. Spot also a qualitative standard mixture (aflatoxins B_1, B_2, G_1, G_2) to check the resolution of the system. Develop with benzene:water:ethanol solvent mixture as in the AOAC procedure for peanuts and peanut products (2).

Examine the plate under UV light (3600Å); if the sample contains aflatoxin B_1, spot 1, 3, 5, and 7 μl of an appropriate dilution (or larger amounts of the extract) and compare the intensity of fluorescence with that of 1, 3, 5, and 7 μl standard B_1 (1 μg/ml) spotted on the same plate.

Preliminary Investigation of Alternative Procedure

Weigh 50 g comminuted sample in a glass flask. Extract 30 min with 100 ml dimethyl sulfoxide:benzene (30:50), using a mechanical shaker. Displace the benzene by adding 70 ml water; cool the flask. Dry a 25 ml aliquot of

Table 1. Recovery of aflatoxin B_1 added to green coffee beans

Aflatoxin Added, μg/kg	Recovery, %
Florisil Method (AOAC (2))	
2	60
	80
5	—
	100
20	70
	70
40	83
	79
50	75
	80
200	70
	—
DSMO:Benzene Extraction Method	
2	100
	80
5	80
	100
20	67
	80
40	100
	100

benzene under nitrogen and chromatograph through Florisil only, as described above. Then dissolve the residue in 500 μl benzene and separate by TLC. The residue represents material extracted from 25 g coffee.

Results and Discussion

Varying amounts of aflatoxin B_1 solution (1 μg/ml) were added to comminuted green coffee beans. The same standard solution was used to compare the intensity of fluorescence on the thin layer chromatograms. The recoveries obtained are summarized in Table 1. Yellow tailing may lead to an overestimation in the intensity of fluorescence when 30 μl extract or more is spotted. The chromatographic pattern obtained with a maximum aliquot of 25 μl extract (500 μl total volume) was still clear and undistorted. Good resolution of the qualitative mixed standard of aflatoxins added to a coffee extract was obtained on the plate with the solvent system used. Eluting the Florisil column with an additional 200 ml acetone still yielded aflatoxin B_1 (corresponding to about 1–5% of the original level added). No attempt was made to determine aflatoxin B_2, G_1, or G_2 with this method.

Some investigators report poor recoveries of aflatoxin B_1 from Florisil columns, contrary to the experience in our laboratory with a single large batch of Florisil which performed satisfactorily. Pesticide residue analysts have reported similar discrepancies in Florisil performance and are now trying to solve the problem by standardizing batches to obtain uniform adsorptivity and elution pattern. A comparison of results obtained by this method and the AOAC method is given in Table 1.

Samples of green coffee, especially selected by four coffee producing plants, were analyzed by this method. The samples had been collected since 1964 and were chosen because they were visibly moldy and had undesirable odors or flavors (e.g., musty, fermented) or contained unusually high levels of defective beans.

The survey included 58 samples of green coffee grown in Angola, Brazil, Cameroun, Colombia, Ivory Coast, Ethiopia, Indonesia, Liberia, Mexico, and Uganda. No aflatoxin was detected in any sample tested.

The method discussed above is lengthy and laborious. A short extraction procedure was investigated and promising preliminary results were obtained.

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Fluorodensitometric Measurement of Aflatoxin Thin Layer Chromatograms

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The precision limit of visual comparison procedures used in aflatoxin assay methods can be no better than ± 20% for a single observation and, under operating conditions, it is probably close to ± 28%. Fluorodensitometric procedures described in the literature can improve this precision to ± 9% for the average of multiple observations. A possible source of inaccuracy in the published densitometric procedures is pointed out and a method using internal standards to avoid the inaccuracy is presented. Tested with spiked extracts of "clean" peanut butter, the procedure gave an average 101 ± 3% recovery of added aflatoxin B_1 and 89 ± 6% recovery of aflatoxin B_2 with a precision for individual assays equal to the precisions reported for the other procedures. The major source of error was shown by elimination to reside in the thin layer chromatography and aflatoxin instability.

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