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Fluorometric Assay for Aflatoxins

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Aflatoxins are widely known as potential carcinogens when they are administered in animals through feeds. The infection of the fungus, Aspergillus flavus or A. parasiticus may produce these toxins in feed grains. Numerous studies are in progress to prevent the infection of the causative organisms. A few investigations are also in progress in an attempt to remove the toxins from grains which have low level (under 400 ppb) contamination (Chakrabarti, 1981). An accurate assay for aflatoxin components is essential for a laboratory either doing research or routine survey analyses. The method that is now widely adopted by the government laboratories for the assay of individual aflatoxin components (B_1, B_2, G_1 and G_2) utilizes a TLC technique (AOAC, 1975). The extraction and clean-up steps of this technique were further researched (Seitz and Mohr, 1976). The method is still time consuming. It is, therefore, very important to develop a rapid and accurate assay technique for aflatoxins. The current research proposes a technique which utilizes a Turner Fluorometer.

Fluorescence properties of aflatoxins were studied (Carnaghan, et. al. 1963; Davis, et. al. 1966; and Diener and Davis, 1966). The fluorescence detection technique was applied to wheat, oat, barley and sorghum and was proven satisfactory (Bothast and Hesseltine, 1975). Measuring aflatoxins on TLC plate using a Turner fluorometer was also attempted (Chen and Friedman, 1966; and Pons, et. al. 1966). A fluorometric measurement of aflatoxins in the range of 0 to 100 ug. was developed on the fluorisil layer of minocolumns (Valesco, 1976).

MATERIALS AND METHODS

Chemically pure aflatoxins were purchased from Aldrich Chemicals, Milwaukee, Wisconsin. Stock solutions were made by dissolving 10 mg. of aflatoxins in 50 ml. of chloroform (99% purity). Aflatoxin B_1 was spotted in 20, 40, 60, 80 and 100 micrograms each 1.5 cm. apart on 250 microns thick silica gel G thin layer chromatography (TLC) plates. Special care was taken so that the spots did not spread over 5 mm. in diameter. The spots were scanned in a Turner fluorometer model 111-003 attached with a

model 2 TLC scanner. The conditions that were maintained for scanning were as follows: Filter No. 7-37 was used as the primary filter whereas No. 48 was used as the secondary filter. The light setting was kept at 3. The slit width that controlled the amount of the incident light on the sample was the only variable in the research. The fluorescence of the aflatoxin concentrations was recorded in peaks. The peak areas were recorded (1/2 base x height). A simple working method for extracting aflatoxins from the native extract was developed. The toxic corn was first extracted with the pH 6 water in the proportion of 50g. corn per 100 ml. of pH 6 water in an American Optical water bath preset at 65°C and shaken at 85 gyrations/minute for an hour. The toxic extract was separated by chloroform in a separatory funnel and the chloroform phase was reduced to 10 ml. by air-drying to be used for spotting on TLC plates. A specific Aflatoxin component was identified by matching with the known. All chromatographic separations were visualized by an UV light in a chromatovue (UV Box). The developing solvent was made of chloroform and acetone mixed in the proportion of 9:1. The visualized spots were circled by a lead pencil and recorded in the fluorometer. The peak areas of the unknown samples were measured from the standard curve. An attempt was also made to establish the linearity between peak areas and the slit width.

RESULTS AND DISCUSSION

The attempt to develop the fluorometric assay yielded some interesting results. The areas of the recorded peaks showed a linear relationship with the rising ppb of aflatoxins within the range of 0 to 100 ppb. Direct proportionality between peak areas and increasing illumination (as controlled by the slit width settings between 0 & 3) was also established for any given concentration of aflatoxin under 100 ppb (Table I). The fluorescent peak areas from the contaminated corn samples did not always fall within the range of the standard curve. On the other hand, the unknown samples could be diluted to the extent that it could be made to fit in the range of the standard curve. In that case, the dilution factors were properly maintained. The data collected as of yet exhibits similar relationship for the other aflatoxin components, such as B_2, G_1 and G_2.

The technique developed in this study reduced the time element as compared to the widely adopted AOAC method but its accuracy can still be questioned as compared to the other one. The peak areas were based on chromatographic separations only. One cannot be absolutely sure if chloroform extracts contained any compound other than aflatoxins (with a close Rf value) and if that has contributed to the fluorescence of the spots. The current technique should be tried in different laboratories prior to the final adoption for routine analysis. The observations were repeatable, however. the minor differences in peak areas that were recorded among repeated trials, could be due to a slight variation in the spread of the spots on TLC plates.

Table 1. Interaction of slit width and aflatoxin concentrations on peak areas

SLIT WIDTH	20/UG/UL	40 UG/UL	60 UG/UL	80 UG/UL	100 UG/UL
0.5	28	32	35	36	40
0.7	48	56	60	68	77
0.9	99	108	127	133	143
1.1	117	122	130	145	165
1.3	139	171	190	219	243
1.5	194	207	221	262	291
1.7	221	227	241	368	331
1.9	269	281	293	322	347
2.1	190	242	263	311	359
2.3	208	281	311	349	369
2.5	311	325	338	349	368

The cost of the instrumentation in this technique is fairly inexpensive and just minimum expertise is required to master the technique. Most important, the entire operation for the analysis of each sample on the average, did not require more than an hour to complete since the TLC plate can hold many samples.

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