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Development of a Fluorescence Polarization Assay for the Determination of Aflatoxins in Grains

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Aflatoxins produced by *Aspergillus flavus* are commonly found in human and animal foods including grains, cereals, peanut products, sorghum, and soy seeds. Exposure to aflatoxins has been associated with carcinogenicity. This paper reports a simple, portable, and rapid fluorescence polarization (FP) assay for aflatoxin determination in grains. This immunoassay is field portable, homogeneous, and without any washing and cleaning steps. The assay is based upon the competition between free aflatoxin and an aflatoxin−fluorescein tracer for an aflatoxin-specific monoclonal antibody in solution. A series of naturally contaminated corn, sorghum, peanut butter, and peanut paste samples were analyzed by FP and compared with HPLC results. Similarly, spiked popcorn samples were analyzed by FP. FP results of naturally contaminated samples correlated well with HPLC ($r^2 = 0.97$). FP analysis of spiked popcorn samples (with a mixture of $B_1/B_2/G_1/G_2$, 7/1/3/1, w/w) gave a good correlation with spiked values ($r^2 = 0.99$). However, FP consistently underestimated the aflatoxin contents. This was perhaps due to low cross-reactivity of the antibody used toward B_2, G_1, and G_2 aflatoxins. These results combined with the portability and simplicity of the assay suggest that the assay can be used for screening total aflatoxin in grains.

KEYWORDS: Aflatoxins; fluorescence polarization

INTRODUCTION

Aflatoxins produced by *Aspergillus flavus* molds were first detected in the late 1960s (*1*−*3*). Different forms of aflatoxin, including B_1, B_2, G_1, and G_2 (**Figure 1**), are found in many forms of human foods, cereals, grains, and peanut products and are known for their toxicity and carcinogenicity (*3*−*6*). Various studies suggested a link of aflatoxin exposure with an increased occurrence of liver and lung cancer (*23*). Some recent studies, however, submit that the aflatoxins may not be the cause for these cancers in humans (*21*, *22*). Nevertheless, there is a great concern about the toxicity of aflatoxins in grains.

Various methods for the determination of aflatoxins in grains include TLC, HPLC, RIA, ELISA, and fiber-optic based immunoassays (*8*−*13*). Whereas chromatographic methods require extended cleanup steps and derivatization after extraction in order to get rid of interfering substances, commercially available ELISAs require enzymatic reactions and washing and separation of bound and free label.

We now report a fluorescence polarization (FP) assay for the determination of aflatoxins in grains. In FP, the signal for aflatoxin detection is directly measured without any further steps after extraction. FP is a powerful and sensitive technology for the determination of the binding of small molecules to larger ones in solutions. This technique has been extensively used for human clinical diagnostics (*14*). It measures the rate of rotation of fluorescent molecules. Small fluorescent molecules rotate quickly and give a low FP value. Large molecules, on the other hand, rotate more slowly and therefore have a higher FP value. A small aflatoxin molecule attached to a fluorophore [aflatoxin-fluorophore (tracer), **Figure 1**] rotates quickly and gives a low FP value (mP, millipolarization). Interaction of this tracer with antibody forms a big immunocomplex that rotates more slowly than the tracer and gives a higher FP value. Depending upon the amount of free aflatoxin in the antibody solution, a competition between free aflatoxin and the tracer occurs and the polarization value changes accordingly (*14*). Some of the common features for FP assays are as follows: separation of bound and free ligand is not required and there are no washing steps; there are no enzymes involved in these assays; and there is essentially no cleaning step. Because FP is dependent upon the rate of rotation of the molecules and not on the total intensity, there is minimal effect by colored and cloudy solutions. FP assays can be performed in milk, blood, and chlorophyll. Our group has extended the use of FP to animal disease diagnostics, food pathogens, and grain mycotoxin determination (*15*−*17*). Recently we reported the use of FP for the determination of fumonisins and deoxynivalenol (DON) in grains (*18*, *19*).

Traditionally, aflatoxins are effectively extracted with a mixture of water and a polar organic solvent. Therefore, during an assay development, an antibody is needed that is resistant to the organic solvents used for extraction. We report the use of such an antibody for FP assay development. Spiked as well as naturally contaminated samples were used to validate the results.

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Naturally contaminated samples were compared with HPLC results performed in a separate laboratory.

EXPERIMENTAL PROCEDURES

Materials and Methods. Reagents were used as received unless otherwise noted. Aflatoxin monoclonal antibody (A-9555) was purchased from Sigma Aldrich Chemical Co. A methanol resistant aflatoxin antibody (mouse, monoclonal) (ABII) was provided by Dr. Chris Maragos, USDA-ARS-NCAUR, Peoria, IL. Preweighed and spiked popcorn samples (0−320 ppb range), naturally contaminated corn, peanut butter, sorghum, and peanut paste samples (along with their HPLC analysis, 0−145 ppb) and aflatoxin stock standard solution (5 ppm) containing aflatoxins B_1, B_2, G_1, and G_2 (7:1:3:1, w/w) were purchased from Trilogy Analytical Laboratory Inc., Union, MO. All solvents were of HPLC grade. Aflatoxin B_1, *O*-carboxymethyl-hydroxylamine-hemihydrochloride, dicyclohexylcarbodiimide (DCC), fluorescenamine isomers I and II, and TLC plates (K6 silica gel, 60A, layer thickness = 250 μm) were purchased from Sigma Aldrich Co. PBSA-BGG buffer (pH ∼7.4) was prepared by dissolving sodium chloride (9.0 g), sodium dihydrogen phosphate monobasic (0.314 g), sodium hydrogen phosphate dibasic (1.1 g), sodium azide (1.0 g), and bovine γ-globulin (BGG, 0.1 g) in 1 L of distilled water.

Preparation of Aflatoxin Tracer. *Aflatoxin B_1 Oxime (**1**).* Aflatoxin B_1 oxime was prepared according to a modification of the literature method (*4*, *5*). Briefly, aflatoxin B_1 (5 mg, 0.016 mmol) and *O*-carboxymethyl-hydroxylamine-hemihydrochloride (41 mg, 0.19 mmol) were mixed with 1.2 mL of absolute ethanol in a 10 mL round-bottom flask fitted with a magnetic stirrer and a condenser. To this solution was added 230 μL of 2 M NaOH (0.46 mmol) with stirring, and the mixture was refluxed for 3 h. The resultant solution was stirred overnight at room temperature, concentrated on a rotary evaporator, and diluted to 1.5 mL with water. Drops of 1 N NaOH were added to adjust the pH to ∼9, and the solution was washed with ethyl acetate (2 × 3 mL portions). The aqueous layer was acidified with 6 M HCl to a pH of ∼2, and the resultant mixture was stored at 2−5 °C in a refrigerator. A precipitate formed, which was separated and air-dried. This solid was dissolved into 500 μL of THF to give a stock solution of oxime product (**1**; **Figure 1**). TLC of **1** on silica using ethyl acetate/MeOH/NH_4OH (32:17:5) gave a major spot at $R_f \sim 0.5$.

*Conjugation of **1** with Fluorescenamine (Isomer II) and Fluorescenamine (Isomer I).* A 20 μL portion of the THF solution of **1** was mixed with a 20 μL CH_2Cl_2 solution of DCC (10 mg/mL) and the mixture diluted to 200 μL in CH_2Cl_2. After 2−3 min, 20 μL of THF solution of fluorescenamine (isomer II, 10 mg/mL) was added. The reaction tube was incubated overnight at room temperature. TLC ($CHCl_3$/MeOH/CH_3CO_2H, 40:10:3) of the reaction mixture showed many spots (*25*). One spot at $R_f \sim 0.7$ (absent in the control) was collected and dissolved in MeOH. This was diluted in buffer appropriately to give an intensity of ∼400000 (at 490/520 excitation/emission) when 10 μL of the diluted tracer **2** was added to 1 mL of buffer. All of the reaction mixture was separated on preparative TLC, collected, and dissolved in methanol to give the stock tracer product (**2**; **Figure 1**). This tracer gave a stable FP value of ∼40 mP. After addition of 10 μL of 1/50 diluted antibody (Mab, A-9555) (overall 1/5000 dilution), the polarization slowly increased to ∼230 mp in 5 min.

The same reaction was done by reacting **1** with fluorescenamine (isomer I**)** using the same quantities and the product (**3**; **Figure 1**) with a similar R_f value of ∼0.7 isolated in a similar way. However, this tracer showed less sensitivity as compared to tracer **2** prepared from isomer II. The starting polarization (∼32 mP) increased only to ∼140 mP upon the addition of antibody A-9555 to the buffer solution.

To confirm the specific reactivity of tracer **2**, 1 mL of buffer was taken and mixed with 10 μL (1/50 diluted) of antibody and 10 μL of aflatoxin B_1 (0.8 mg/mL) to observe an inhibition of the reaction due to free aflatoxin in the solution. After standing at room temperature for 5 min, it was blanked in the machine,and after the additon of tracer, the polarization was found to be stable at ∼41 mP.

Reactions of **1** with other available amine derivatives of fluorescein including 5-aminoacetyl-amidofluorescein (5AAF), 5-(5-aminopentyl-thioureidyl fluorescein (5,5APTF), fluorescein thiosemicarbazide (FTSC), 4-aminomethyl fluorescein (4AMF) (no active product), and 5-aminomethyl fluorescein (5AMF) (no active product) were similarly carried out. However, the respective products after purification did not yield tracers with reasonable sensitivity to aflatoxin antibodies. The product of **1** with 5AAF gave a starting mP of 30 that increased to ∼65 upon the addition of diluted antibody (A-9555). Similarly, the reaction product of **1** with 5,5APTF gave a starting mP of ∼35 that increased to 102 upon the addition of properly diluted antibody. Reactions of **1** with FTSC, 4AMF, and 5AMF did not give any products sensitive to aflatoxin antibodies.

Apparatus. The instrument employed was the Sentry FP, a portable machine (Diachemix Corp.). The instrument receives power from the battery of an attached laptop computer. All of the disposables including culture tubes and pipet tips were purchased from VWR Scientific. Standard values were plotted using Sigma plot (Graph Pad Prism).

FP Assay Development. Two antibodies were used for assay development. Initially the kinetic mode was used to find the equilibrium time where a stable polarization (mP) value is obtained. One milliliter of diluted antibody solution [A9555 (1/250000 dilution), ABII (1/150000 dilution)] was mixed with 40 μL of free aflatoxin B_1 solution. After a blank measurement in the instrument, 10 μL of previously diluted tracer (with an intensity of ∼400000) was added and the polarization change monitored in the machine for 10 min.

Effect of Methanol Concentrations on the Stability of Aflatoxin Antibodies (A-9555 and ABII) in FP. One milliliter of diluted antibody solution (A-9555, 1/250000) in PBSA−BGG was mixed with varying amounts of methanol (0, 10, 20, 30, 40, and 50 μL, respectively). After a blank had been measured, tracer was added and polarization measured for a specific time in each case.

Solid ABII was reconstituted by dissolving in PBSA (1 mg/mL) and diluted to a final concentration of 1/150000 in PBSA−BGG. One milliliter of this solution was mixed with varying amounts of methanol (0, 10, 20, 30, 40, and 50 μL, respectively), and polarization values were measured similarly to the method used for A9555.

FP Analysis of Aflatoxin in Naturally Contaminated Samples Using ABII. A series of aflatoxin standards were prepared in MeOH/water (70/30) by diluting a stock solution that contained B_1, B_2, G_1, and G_2 (7/1/3/1, w/w ratio). Twenty grams each of naturally contaminated corn, sorghum, peanut paste, and peanut butter samples was extracted with a 100 mL mixture of MeOH/water (70/30) in duplicate by shaking each sample from time to time for 0.5 h. Extracts were filtered through a fine filter paper (VWR Scientific, quantitative, 494, 12.5 cm) and stored in sealed bottles at room temperature for analysis. Standard or sample (40 μL) was mixed with 1 mL of antibody solution (1/150000 in PBSA−BGG) in a test tube. After each sample had been blanked in the batch mode in duplicate, 10 μL of diluted tracer (**2** with an intensity of ∼400000) was added to each tube. After 15 min of incubation at room temperature, the FP was measured for each tube using their respective blank values. A standard curve was plotted using duplicate standard values, and the concentration of samples was calculated from the graph.

HPLC Analysis of Aflatoxins in Naturally Contaminated Samples. HPLC analysis was performed by Trilogy Analytical Laboratory, Inc., using AOAC Official Method 994.08 (*24*) with the following modifications: 20 g samples were extracted by blending for 3 min with acetonitrile/water (84/16). The extracts were filtered through a quantitative filter paper and purified through solid phase cleanup columns (Puritox, column TC-A100, Trilogy). Purified extracts were diluted with water (200 μL of extract to 800 μL of water), and 100 μL was injected into the HPLC system containing postcolumn brominating KOBRA cell (instead of precolumn TFA as in the AOAC method) (*24*). Corn samples were run in triplicate, whereas peanut butter, sorghum, and peanut paste samples were run in duplicate. Aflatoxins B_1, B_2, G_1, and G_2 were analyzed and averaged separately. Further HPLC conditions were as follows: mobile phase, combine 2500 mL of water with 500 mL of methanol and 500 mL of acetonitrile, add 1.2 g of potassium bromide and 360 μL of nitric acid, sonicate for at least 10 min or filter through glass fiber filter to remove air bubbles; flow rate, 2 mL/min; fluorescence detector, emission at 440 nm, excitation at 360 nm; approximate retention times, G_2 = 5 min, G_1 = 6.2 min, B_2 = 7.2 min, and B_1 = 10 min.

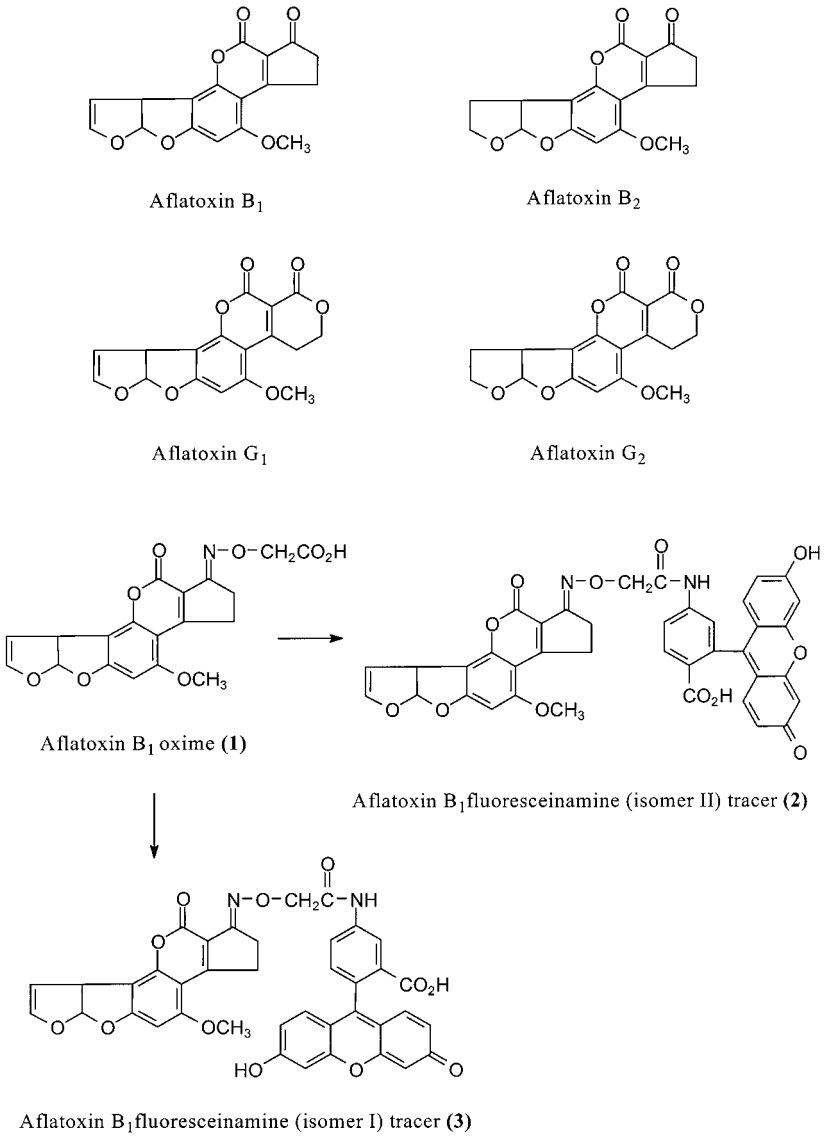

Figure 1. Chemical structures of aflatoxins B_1, B_2, G_1, and G_2, aflatoxin B_1-oxime (**1**), aflatoxin B_1–fluorescein tracer (**2**), and aflatoxin B_1–fluorescein tracer (**3**).

A standard was placed after every fifth sample to calculate the recovery. Any recovery of the standard below 90% meant the whole run was repeated.

FP Analysis of Aflatoxin in Spiked Popcorn Samples Using ABII. Twenty grams of crushed samples of aflatoxin-free popcorn was preweighed and spiked with various parts per billion levels of aflatoxins (0−320 ppb) ($B_1/B_2/G_1/G_2$, 7/1/3/1, w/w). These samples were extracted using a 100 mL mixture of MeOH/water (70/30) by shaking each sample from time to time for 0.5 h. Extracts were filtered through a fine filter paper (VWR Scientific, quantitative, 494, 12.5 cm) and analyzed. FP analysis was performed in quadruplicate on each extract, and values are reported as an average of the quadruplicate analysis.

Cross-Reactivity of Aflatoxins B_2, G_1, and G_2 with Respect to B_1. Aflatoxin solutions of various concentrations were prepared for different analogues and assayed in a standard way. Experimental concentrations of B_1, G_1, and G_2 were calculated from the standard graph of aflatoxin B_1. IC_{50} was calculated as the concentration of respective aflatoxin analogue that gives an FP response of 50%, where 100% corresponds to the response of the assay without aflatoxin and 0% corresponds to the response without antibody. Percent cross-reactivity was calculated by dividing the IC_{50} of B_1 by the IC_{50} of respective aflatoxin species.

RESULTS AND DISCUSSION

In FP assays, a sample is mixed with an antibody solution in a test tube and used as a blank in the FP machine. An aflatoxin tracer is added and, after a few minutes of incubation at room temperature, the FP signal is measured. A change in the FP value is proportional to the free aflatoxin concentration present in the solution. Due to the high affinity of antibodies, results in these assays are highly dependent upon the amount of antibody added and the time of addition of tracer in the tube before the sample is read. To control these two factors, (i) the antibody stock solution was diluted and each time 1 mL of solution was used from the same stock, and (ii) the assay was performed in the batch mode instead of the kinetic mode. In this mode, standards and samples were mixed with 1 mL of antibody solution each in separate test tubes, and their blank readings were taken one by one. After a fixed amount of tracer (10 μL) had been added in each tube, they were incubated at room temperature for a fixed time (15 min) and read in the instrument against their respective blank values. This also reduces any interference due to background fluorescence in the samples.

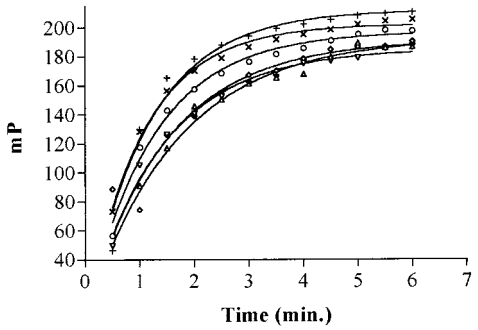

Figure 2. Effect of MeOH on FP assay for aflatoxin determination using antibody A-9555: (+) 0 μL of MeOH; (×) 10 μL of MeOH; (○) 20 μL of MeOH; (◇) 30 μL of MeOH; (▽) 40 μL of MeOH; (△) 50 μL of MeOH.

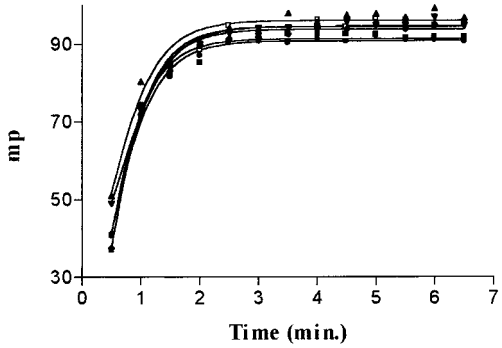

Figure 3. Effect of MeOH on FP assay for aflatoxin determination using antibody ABII: (■) 0 μL of MeOH; (▲) 10 μL of MeOH; (▼) 20 μL of MeOH; (◆) 30 μL of MeOH; (●) 40 μL of MeOH; (□) 50 μL of MeOH.

During our assay development, we found that antibodies A9555 and ABII are good candidates for studies. To find an equilibrium time for a stable polarization value (mP), initial studies were carried out in kinetic mode. These studies suggested that it takes ~10 min for the mixtures to get the maximum binding of final immunocomplex. This is very common when high-affinity antibodies are used for FP studies. These studies suggested that the tracer−antibody combination of aflatoxin−fluorophore (**2**; **Figure 1**) and any one of these antibodies was suitable for assay development.

Because aflatoxin is extracted using a mixture of organic solvent and water, antibodies (A9555 and ABII) and tracer (**2**; **Figure 1**) were tested for their stability in various quantities of methanol. Our studies show that antibody A9555 decayed in organic solvents, where a decrease in polarization value was observed with an increase in the concentration of methanol (**Figure 2**). We have observed that the tracer itself is stable in methanol and related organic solvents for an extended period of time. This slow decay of antibody A9555 with time and methanol concentration ultimately leads to an uncertainty in the results. Therefore, antibody A9555 was not further studied. ABII was similarly tested for its stability toward methanol. The results showed the consistent stability of this antibody toward various methanol concentrations (**Figure 3**). Therefore, ABII was used for further analysis after it had been diluted 1/150000 in PBSA−BGG.

FP Analysis of Naturally Contaminated Samples; Comparison with HPLC Analysis. Samples from naturally contaminated grains [corn (11 samples), sorghum (4 samples), peanut butter (4 samples), and peanut paste (2 samples)] were extracted in duplicate using methanol/water (70/30). Each extracted sample was analyzed in duplicate by FP (with ABII). An average of these four values was taken and compared with an average value of HPLC results. In HPLC analysis, samples were extracted with acetonitrile/water (84/16); corn samples were run in triplicate, and all other samples were run in duplicate. Because FP calculates total aflatoxin concentration, HPLC results were also calculated as an average of total aflatoxin for comparison purposes. The data in **Table 1** show the aflatoxin levels of extracted samples both by FP and by HPLC in nanograms per milliliter (ppb). The results indicate a strong correlation between the two methods ($R^2 = 0.97$, slope = 1.19, X intercept = 3.45). Because the samples were naturally contaminated, and HPLC is used as a standard for analysis, no other method was used to find the extraction efficiency of FP. The overall good agreement between FP and HPLC assays, performed at different locations, suggests that the assay can be used for screening various grains for aflatoxin analysis. The overall simplicity of the FP test coupled with no cleaning and washing steps and portability makes the test very attractive.

Table 1. Comparison of Aflatoxin Analyses of Naturally Contaminated Samples Using FP and HPLC[a]

	FP (total aflatoxin)		HPLC (total aflatoxin)	
sample	av	SD	av	SD
corn				
1	0	0	0.50	0.10
2	9.00	2.70	10.20	1.67
3	69.14	6.57	56.46	0.57
4	47.05	1.35	41.50	1.08
5	32.75	5.89	33.25	6.25
6	106.86	4.53	145.20	9.86
7	75.05	2.42	75.00	6.10
8	55.96	4.47	47.13	4.72
9	71.16	1.28	60.83	2.40
10	17.38	1.69	23.00	3.53
11	5.40	1.00	12.01	2.42
sorghum				
1	26.54	1.52	27.20	2.10
3	14.04	7.79	18.70	1.80
4	5.53	2.60	5.50	0.70
5	2.68	1.19	0	0
peanut paste				
10	9.23	2.86	10.80	2.80
25	26.11	1.21	29.30	1.60
peanut butter				
2783-2	6.62	1.62	8.90	1.20
2193-2	8.61	1.01	12.50	2.50
2508-3	4.92	0.93	6.50	1.44
2279-5	12.56	2.63	14.90	0.74

[a] For FP analysis, samples were extracted in duplicate and each extract was run in duplicate. Average aflatoxin (av) plus standard deviation (SD) is therefore an average of four values. For HPLC analysis, corn samples were extracted and analyzed in triplicate, whereas all other samples were analyzed in duplicate. In HPLC, B_1, B_2, G_1, and G_2 were analyzed separately, and after determination of total aflatoxin in each run, their average was taken for comparison.

Analysis of Spiked Popcorn Samples. Ten samples (20 g each) of crushed popcorn were spiked with various concentrations of aflatoxins [$B_1/B_2/G_1/G_2$, 7/1/3/1 (w/w)] (0−320 ppb). These samples were extracted with a 100 mL solution of methanol/water (70/30). The extracted samples were analyzed in quadruplicate using ABII. FP results (**Table 2**) show a good correlation between theoretical and FP values (R^2 =0.996, slope = 1.70, X intercept = 3.14). However, FP results consistently underestimated the values. There are various possible explanations for these results. The fact that FP results matched well with HPLC results for naturally contaminated samples of different commodities may not apply to any correlation in spiked popcorn samples. Perhaps HPLC also underestimates the results (although it may also overestimate sometimes). It has been noted especially for peanut samples, for which addition of hexane has

Table 2. FP Analysis and Recovery of Aflatoxins with Respect to Spiked Values in Popcorn Samples[a]

ppb (spiked)	ppb (FP)	SD	recovery (%)
0	0.47	0.30	
10.00	5.79	1.64	57.90
10.00	6.43	2.16	64.30
20.00	14.60	1.56	73.00
20.00	13.16	0.93	65.80
30.00	23.30	0.55	77.60
40.00	31.12	2.32	77.80
80.00	57.72	4.84	72.10
160.00	94.74	3.44	59.20
320.00	189.00	8.65	59.00

[a] Samples were run as duplicates of duplicates (SD = standard deviation of average of four values).

Table 3. Cross-Reactivity Results of Aflatoxins B_1, G_1, and G_2 toward Antibody ABII Using a Standard Curve Prepared with Pure Aflatoxin B_1[a]

B_2		G_1		G_2	
ppb (calcd)	ppb (added)	ppb (calcd)	ppb (added)	ppb (calcd)	ppb (added)
3.14	10	3.75	10	3.37	10
10.64	25	9.06	25	7.76	25
14.65	40	16.44	40	17.56	40
33.04	80	25.16	80	25.16	80
40.94	100	26.61	100	27.35	100

[a] Average IC_{50}: B_1 = 28 ng/mL, B_2 = 90 ng/mL, G_1 and G_2 = 100 ng/mL. % Cross-reactivity of B_2 = 31% and of G_1 and G_2 = 28% with respect to B_1.

recently been suggested for proper extraction (*20*). Addition of hexane, however, may be merely to take care of oily material in peanut butter. This oil does not interfere in FP analysis, where blood and milk samples are used routinely without any interference (*14*). It has also been suggested that mere shaking may not be enough for aflatoxin recovery. This, however, is not clear because in naturally contaminated samples, two different laboratories used two different extraction methods (in HPLC analysis, samples were blended and filtered, whereas in FP analysis, samples were shaken and filtered) and still gave a good correlation of results.

There is one major difference in naturally contaminated and spiked samples. Naturally contaminated samples had mainly aflatoxin B_1 and some B_2, but almost no G_1 and G_2, whereas popcorn samples were spiked with a mixture of $B_1/B_2/G_1/G_2$ (7/1/3/1, w/w). Due to the difference in cross-reactivity of aflatoxin B_1, B_2, G_1, and G_2 analogues toward antibody (**Table 3**), a difference in analysis is expected. We investigated the cross-reactivity of these aflatoxins individually toward said antibody. These aflatoxins were separately diluted in a mixture of methanol/water (70/30) to give a range of concentrations. They were analyzed against a standard curve using pure B_1 standards. Results show that B_2, G_1, and G_2 all cross-react with the antibody by ~30% (**Table 3**; IC_{50} ~28 ng/mL for B_1, ~90 ng/mL for B_2, and 100 ng/mL for G_1 and G_2) (*18*). This strongly suggests that low results in spiked corn samples were due to the low cross-reactivity of B_2, G_1, and G_2 collectively toward the antibody as compared to B_1. By calculating the theoretical recovery based upon the cross-reactivity of these aflatoxins toward said antibody, 1 ppb of B_2, G_1, and G_2 will give only 0.3 ppb response in this assay. Considering this 30% cross-reactivity of B_2, G_1, and G_2 toward the said antibody, the theoretical recovery of ~57−78% corresponds to ~80−110% if there was only B_1 present. This seems to be a weakness of the FP assay, and further research is warranted. Because G_1 and G_2 are not present to a significant extent in many common grain commodities except peanut butter, this low cross-reactivity of the antibody toward aflatoxin derivatives other than B_1 does not seem to be a major factor for assay development. Further studies are in progress to improve the sensitivity, repeatability, and recovery of various aflatoxin derivatives.

In conclusion, we report the development of a rapid, simple, and sensitive FP assay for the determination of aflatoxins in various grains. The assay is field portable and homogeneous and does not require any cleaning and washing steps. Reagents are very stable and require no special handling. In the present form, the assay is a good tool as a screening method for aflatoxin analysis (in the range of 5−200 ppb) where a portable instrument is run without the need of a centralized laboratory.

SAFETY

Aflatoxin compounds have been related to carcinogenicity and animal disease and should be handled with appropriate caution. Standard safety measures (gloves and laboratory coat) should be used when one is working with aflatoxin samples. Extraction and waste disposal should be done according to standard environmental procedures.

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