Fluorescence Polarization as a Means for Determination of Fumonisins in Maize

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Fumonisins, mycotoxins produced by certain species of *Fusaria*, are commonly found worldwide as contaminants in maize. This paper reports the development of a rapid, portable fluorescence polarization-based assay for fumonisins in maize. The assay was based on the competition of unlabeled fumonisin, from a sample, with a fluorescently tagged fumonisin (FB_1-FL) for a fumonisin-specific monoclonal antibody in solution. The fluorescence polarization (FP) of the tagged fumonisin was increased upon binding with the antibody. In the presence of free toxin, less of the FB_1-FL was bound and the polarization signal was decreased. The assays were very simple to perform, requiring only mixing of an aqueous extract of maize with the tagged fumonisin and antibody, and required <2 min per sample, excluding extraction time. Two permutations of the assay were tested, one with each sample matrix serving as its own blank, and the other with all of the samples compared relative to a PBS blank with normalization of the data similar to an ELISA. The limit of detection, defined as the toxin content associated with a fluorescence polarization signal 5 standard deviations from that of a fumonisin-free control, was 0.5 μg of FB_1/g in spiked maize. Recoveries from spiked maize over the range of 0.5−20 ppm averaged 94.3 ± 13.8%. Forty-eight samples of field-contaminated maize were tested by the FP and an established HPLC method, with a good correlation between the two (r^2 = 0.85−0.88). For these samples, the two variations of the FP assay also compared well to one another (r^2 = 0.97), suggesting the assay principle is very robust. The results, combined with the speed and ease of use for the assay, suggest that this technology has substantial potential as a screening tool for mycotoxins in foods.

Keywords: *Mycotoxin; fumonisin; fluorescence polarization; maize*

INTRODUCTION

The fumonisins are a class of mycotoxins produced by certain species of *Fusaria* in foods. They are characterized by two tricarballylic acid side chains esterified to a 20-carbon backbone having one or more hydroxyl groups and a single primary amine. In addition to the most common member of the family (fumonisin B_1), there are several members lacking one or more of the hydroxyl groups on the backbone (fumonisins B_2, B_3, and B_4), as well as members lacking one or both of the side chains. The fumonisins were first isolated in 1988 (*1*) and since that time have been determined to cause leukoencephalomalacia in horses, pulmonary edema in swine, and cancer in rats (*2*−*5*). The fumonisins have received considerable attention because of their common occurrence in corn and corn-based food and feeds [summarized by Marasas (*6*)]. The extent to which fumonisins pose a hazard to human health is currently being assessed by the U.S. Food and Drug Administration, in part through testing of purified FB_1 in rodents (*7*).

The interest in fumonisins has spurred the development of a variety of analytical methods for their measurement. The fumonisins lack a strong chromophore or fluorophore and are often labeled with one for detection. Commonly used methods include high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and enzyme-linked immunosorbent assay (ELISA). Chromatographic methods for fumonisins were recently reviewed by Shephard (*8*). The most commonly used methods involve extraction with mixtures of acetonitrile/water or methanol/water, isolation of the fumonisins using solid phase extraction or affinity columns, derivatization with a fluorescent marker, and separation by HPLC. Methods that do not require derivatization, such as HPLC with evaporative light scattering detection, have also been described (*9*, *10*). Other methods of analysis include mass spectrometry (*9*, *11*, *12*) and capillary electrophoresis [CE; summarized by Maragos (*13*)].

Immunochemical assays, such as ELISAs, have been developed extensively for detection of the intact and hydrolyzed fumonisins (*14*−*23*). The most sensitive assays, with limits of detection in the low parts per billion range, have relied upon polyclonal rather than monoclonal antibodies (*18*, *22a*). Although acetonitrile/water and methanol/water are still the most commonly used extraction solvents, simple aqueous buffer solutions may be adequate for use with immunoassays (*23*). Most ELISA protocols for small molecular weight materials such as mycotoxins require no sample cleanup other than filtration and dilution, but they do require a washing step to remove any nonspecifically attached label. Excellent reviews of recent immunochemical

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10.1021/jf0010782 CCC: $20.00
Published on Web 01/06/2001

methods for fumonisins include those by Chu (*24*) and Dietrich et al. (*25*). Immunoassay formats other than traditional ELISAs have also been developed for mycotoxins. These include an immunoblot assay for fumonisins, aflatoxins, and zearalenone (*26*) and an immunofiltration assay (*27*). Recently attempts have been made to combine the specificity of immunoassay with the separation power of CE for fumonisins (*28*), and a fiber-optic biosensor has also been demonstrated (*29*). In the latter two publications the fumonisin content was determined from the competition between FB_1 labeled with fluorescein and unlabeled fumonisin for attachment to specific antibodies.

Although the TLC, HPLC, CE, and immunosensor methods have typically relied upon measurement of fluorescence intensity, the signal intensity is only one of several characteristics of fluorescence that can be measured. Others include effects upon excitation and emission spectra and effects upon polarization. Fluorescence polarization (FP) instruments measure the rate of fluorophore rotation in solution rather than fluorescence intensity. The polarization value is independent of the fluorophore concentration and intensity and has the advantage that it is minimally affected by solution opacity or color. Polarization is, however, affected by the size of the molecule, with smaller molecules having higher rates of rotation and lower polarization. Interaction of an antibody with a toxin−fluorophore conjugate increases the effective size of the fluorophore through the formation of an immunocomplex. The increase in size reduces the rate of fluorophore rotation and increases the polarization over that of the unbound fluorophore. In the presence of free toxin the formation of the toxin−fluorophore immunocomplex is suppressed, increasing the rate of rotation and reducing polarization. Therefore, similar to many ELISA methods for small molecules, the signal is inversely proportional to toxin content.

The use of fluorescence polarization in immunoassay was first described ~40 years ago (*30*, *31*) and is currently undergoing a renaissance as a tool for modern analysis (*32*). Several applications were developed in the 1970s [see Dandliker et al. (*33*)]; however, extensive application development did not occur until a commercial instrument was produced in the early 1980s (*34*). The technology has been used for a variety of assays, predominantly in the clinical area, where there are applications for proteins, antibodies to infectious diseases, an equine virus, prescription and illicit drugs, and herbicides. Fluorescence polarization immunoassay was recently reviewed by Nasir and Jolley (*35*). Extension of the technology to simultaneous determination of multiple analytes, using multiple wavelengths, has also been attempted (*36*).

The objective of the present research was to investigate the use of FP for analysis of mycotoxins in maize. Two methods were developed that differed in how the FP data were collected and analyzed (Figure 1): the first involved using each sample as its own blank to minimize matrix effects (how this can be done is explained below); the second involved using a single PBS blank and mathematical normalization to control for matrix effects. These methods are the first published applications of fluorescence polarization to mycotoxin analysis.

MATERIALS AND METHODS

Reagents. Fumonisin B_1 (FB_1) was a gift from Glenn Bennett (USDA-ARS-NCAUR, Peoria, IL). Murine monoclonal anti-fumonisin antibodies were derived from a clone, reference number P2A5-3-F3, described previously (*37*). This antibody was generated against an FB_1−cholera toxin conjugate, reacted with FB_1 (100%, by definition), and cross-reacted with FB_3 (50%) and FB_2 (38%) when assessed in a competitive direct ELISA format (*37*). Bovine γ-globulin (BGG) was obtained from Sigma Chemical Co. (St. Louis, MO).

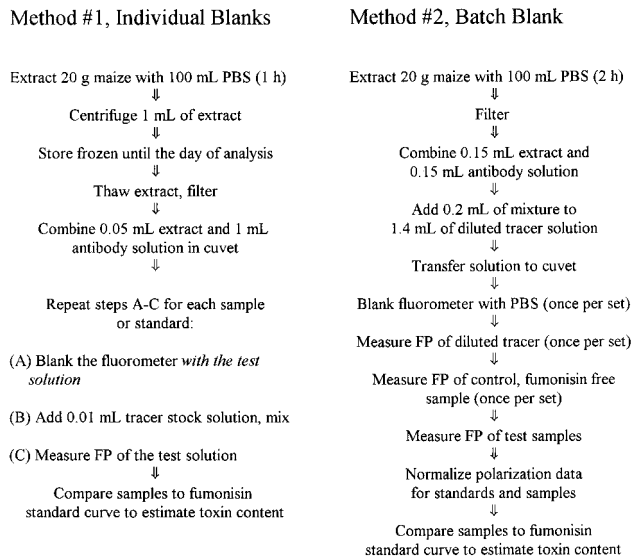

Figure 1. Two fluorescence polarization methods for fumonisins in maize.

Fumonisin Tracer. The primary amine of FB_1 was labeled with 6-[{4,6-dichlorotriazin-2-yl}amino] fluorescein hydrochloride (6-DTAF) at Diachemix Corp. (Grayslake, IL) to produce a tracer, FB_1-FL, that was used in all of the experiments. FB_1 (1 mg in 0.1 mL of DMF) was mixed with 0.1 mL of 1 M sodium carbonate buffer, and 6-DTAF (1 mg in 0.1 mL of DMF) was added. The reaction mixture was shaken and incubated overnight at ambient temperature. The crude product was purified on a Sephadex G-25 column using 0.01 M sodium phosphate (pH 7.5) as the eluant. The first 2 mL fraction was discarded, and the second bright green fluorescent fraction, 5 mL, was collected. This solution gave a fluorescent intensity equivalent to 1 nM fluorescein when diluted 1:20000 in PBS. The purified tracer gave a single spot (R_f 0.3) when tested with normal phase TLC using a chloroform/methanol/acetic acid (30:5:0.5) mobile phase. The stock solution was stored at 2−8 °C, shipped to the National Center for Agricultural Utilization Research (Peoria, IL), then subdivided into 0.1 mL aliquots, and stored at −70 °C until the day of use. Controlled studies of the stability of the tracer have not been conducted; however, anecdotal evidence suggested the tracer remained useful for several months when stored at 4 °C.

Apparatus. The FP instrument was a Sentry FP (Diachemix Corp.). The unit is portable, with power provided from the battery of an attached laptop computer (Gateway, North Sioux City, SD) through a PCMCIA card model DAQCard-500 (National Instruments Corp., Austin, TX). The unit also has only one moving part: an access door into which the sample cuvette, a cell culture tube, is placed.

Maize Samples. Maize was inoculated with one of several strains of *Fusarium moniliforme* as part of field trials investigating the effect of fumonisin production upon the virulence of this fungus. The maize was grown on test plots in Illinois and harvested in the fall of 1999, and samples of whole kernel corn were collected for subsequent analysis. Whole kernel samples were ground using a Romer mill (Romer Laboratories, Union, MO); a portion was then extracted with acetonitrile/water (1+1 v/v) for HPLC assay while a replicate portion was extracted with phosphate-buffered saline (PBS; 10 mM sodium phosphate and 0.15 M sodium chloride in water, pH 7.2) as described below for the FP assay. Acetonitrile/water extracts were applied to strong anion exchange solid phase extraction

columns to isolate the fumonisins, which were then derivatized with *o*-phthaldialdehyde (OPA) for subsequent separation by HPLC with fluorescent detection (*38*). Results of the field trials will be described elsewhere (Dr. Anne Desjardins, USDA-ARS-NCAUR, Peoria, IL, personal communication). Control maize, containing <0.1 μg of fumonisin/g, was used for spiking studies.

Sample Extraction. For FP assay method 1, 20 g of ground maize was mixed with 100 mL of PBS and periodically shaken for 1 h. One milliliter of the aqueous extract was then transferred to a 1.5 mL microcentrifuge tube, centrifuged for 5 min in a minicentrifuge (Bel-Art Products, Pequannock, NJ), and stored frozen until the day of assay. It was then thawed and filtered through a 0.2 μm membrane (25 mm, Gelman Laboratories). For method 2, samples were extracted for 2 h on a Burrell wrist action shaker (Burrell, Pittsburgh, PA) at ambient temperature. The solution was filtered through a 24 cm Whatman 2V filter (Whatman International Ltd., Maidstone, U.K.). The filtrate was used without further preparation. For method 2 the extracts were not frozen, and samples were tested the same day as the extraction. For both methods, samples containing high levels of fumonisins (>25 ppm) were diluted and reanalyzed to ensure data were collected in the optimum range of the FP assay.

FP Assay, Method 1. FB_1 standards were prepared by diluting the FB_1 stock solution with PBS with 0.1% sodium azide (PBSA). One milliliter of antibody solution (3.75 μg) containing 0.1 mg of BGG in PBSA (PBSA-BGG) was placed into a 10 mm × 75 mm glass culture tube (VWR Scientific, West Chester, PA). Fifty microliters of fumonisin standard, or sample extract, was added, and the test solution was mixed thoroughly. The test solution was then placed in the fluorometer and used as the blank. The tracer, 10 μL of FB_1-FL stock solution, was then added and mixed. The test solution containing tracer was then returned to the fluorometer and the FP signal (mP) measured. Data were acquired using Sentry FP software (Diachemix Corp.). Each FP measurement took 5 s, and readings 3−7 were averaged. In this manner each sample matrix served as its own blank before the addition of the tracer. The fumonisin content of unknown samples was estimated relative to response of the FB_1 standards in PBSA.

FP Assay, Method 2. To determine the effects of matrix upon the performance of the FP assay, initial experiments were with FB_1 standards prepared in PBS or control (fumonisib-free) maize extracts spiked over the range of 0.002−20 μg/mL. Spiking the extracts over this range corresponded to a range of FB_1 from 0.01 to 100 μg/g in maize given the 5:1 (v:w) extraction ratio that was used. Control maize was also spiked with FB_1 over the range of 0.01−100 μg/g by adding small volumes of FB_1 stock solutions (0.2−0.4 mL of stock in acetonitrile/water 1+1) directly to 20 g of ground maize before extraction with PBS. Finally, 20 g samples of field-inoculated maize were extracted with PBS as described above.

For assay, 0.15 mL of filtered sample extract was mixed in a polypropylene tube with 0.15 mL (9.4 μg) of antibody in PBS. Separately the diluted tracer solution was prepared by adding 67 μL of tracer stock to 1333 μL of PBS, and then 200 μL of the sample/antibody mixture was added. The solution, having a total volume of 1.6 mL, was transferred to a glass culture tube and placed into the instrument for measurement of FP. To represent all of the data on a scale between 0 and 1, the raw data, in millipolarization units (mP), were transformed using the following equation: $Y_{obs} = (mP_{obs} - mP_0)/(mP_1 - mP_0)$, where mP_{obs} is the signal observed from the test sample, mP_0 is the signal from a control that does not contain antibody, mP_1 is the signal from a control that contains no toxin, and Y_{obs} is the normalized result for the test sample. Simply, mP_0 is the defined minimum signal (i.e., defined "0") and mP_1 is the defined maximum signal (i.e., defined "1") with the test samples at various toxin levels observed between these two extremes. This is analogous to the normalization of absorbance data from ELISAs, which are often expressed as "percent maximum absorbance" or "percent inhibition" to adjust for different color development in the toxin-free control. For estimation of recovery of fumonisin from spiked maize the FP response from spiked maize samples was compared to a standard curve of FB_1 in PBS. The fumonisin content of field-contaminated maize samples was estimated relative to a standard curve of FB_1 prepared in control maize extracts (TableCurve software, Jandel Scientific, San Rafael, CA).

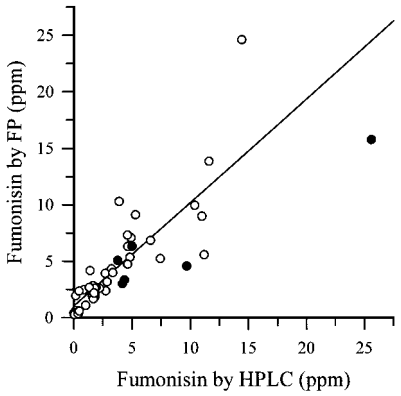

Figure 2. Comparison of HPLC and fluorescence polarization method 1 for determination of fumonisin in contaminated maize. Data are for 48 maize samples analyzed by either HPLC of the OPA derivatized fumonisins (FB_1, FB_2, and FB_3 combined) or total fumonisin as reported by the FP assay. The line is a regression of the data with the equation [fumonisin by FP] = 1.042 + 0.917[fumonisin by HPLC], with a fit of r^2 = 0.854. Solid circles are from six samples that contained more FB_2 than FB_1 by HPLC.

RESULTS AND DISCUSSION

Response to Standards and Field-Inoculated Maize, Method 1. Initial experiments focused on determining the effect of antibody and toxin (in buffer) upon the fluorescence polarization signal from a fumonisin B_1−fluorescein tracer (FB_1-FL). In the absence of antibody the tracer typically produced a signal of ∼60−65 mP. When antibody was present, the signal was increased dramatically, with a 5000-fold dilution of antibody yielding a signal of ∼230−240 mP. This indicated that the antibody was interacting with the tracer sufficiently to affect its rotation. The difference between the two signal extremes, ∼170 mP, was the signal range in which the assays were conducted. When FB_1 was also present in the mixture, the unlabeled toxin competed with the FB_1-FL for the limited amount of antibody. As the FB_1 concentration was increased the proportion of tracer bound to antibody, and therefore the signal, decreased. At high levels of FB_1 (20 μg/mL) the unlabeled toxin effectively minimized interaction of the tracer with the antibody and the signal returned to very near the level of the antibody-free sample.

Comparison of HPLC and FP Method 1 with Field-Inoculated Samples. A comparison was made between FP method 1 and an established HPLC method for the fumonisins. Forty-eight samples of maize from field trials that were naturally and artificially inoculated with *Fusaria* were tested by both methods. The majority of the samples tested were in the range of 1−10 μg/g. Samples were split and a portion extracted with acetonitrile/water for analysis by HPLC and a separate portion extracted with PBS for analysis by FP. The HPLC data are an estimation of "total" fumonisin content obtained by summing the individual contents of fumonisins B_1, B_2, and B_3.

The HPLC and FP assays, which differ widely in the physical basis by which they measure fumonisins, nevertheless showed good agreement (Figure 2). The

linear regression was of the form [fumonisin by FP]= 1.042 + 0.917[fumonisin by HPLC], with a fit of r^2 = 0.854. The monoclonal antibody (Mab) upon which the FP assays were based has been shown previously to cross-react with FB_3 (50%) and FB_2 (38%) when tested in an ELISA format (*37*). Interestingly, in the FP format the cross-reactivity of the same antibody was greater for both FB_3 (77%) and FB_2 (70%). Although this antibody detected FB_2 and FB_3 less efficiently than FB_1 in most cases, this may be of little relevance because FB_1 is almost always the predominant fumonisin found in naturally contaminated maize. Of the 48 samples, 6 were from plants inoculated with a strain of *F. moniliforme* that usually produces greater amounts of FB_2 than FB_1, and these 6 generally gave a poorer response in the FP assay than in the HPLC assay, where they were quantitated separately. From this result we expect that maize samples containing substantially more FB_2 or FB_3 than FB_1 will be reported with artificially low values. The problem is likely compounded by the use of an aqueous solution for extraction because FB_2 and FB_3 are less polar than FB_1 and may be less efficiently extracted from contaminated corn.

The intercept of the regression line indicates that a sample having no fumonisin by HPLC would, on average, give a response of ~1 ppm with this FP method. The effect of the highly contaminated samples (samples containing >8 ppm total fumonisin) and the samples containing predominantly FB_2 upon the shape of the regression line was examined. When these samples were removed from the calculation, the new regression equation assumed the form [fumonisin by FP] = 0.83 + 1.06-[fumonisin by HPLC], with a fit of r^2 = 0.67. Although this marginally improved the intercept, the correlation with HPLC (0.67) was actually worse than when all of the data were included (0.85).

Response to Standards and Spiked Maize, Method 2. To determine the extent of matrix effects and the impact of mathematical normalization of FP data upon the assay, a second method was developed and compared to the first. Method 2 differed from method 1 in two main respects: first, the data were collected using a single PBS blank, rather than blanking each sample before addition of the tracer. Second, two controls were included: FB_1-FL with and without Mab. The data were then scaled (normalized) to the numeric range of 0−1 using the data from the controls. The response of FP method 2 was tested four ways: through FB_1 in buffer (PBS), through FB_1 added to extracts of control maize (spiked extracts), through FB_1 added to control maize (spiked maize), and through a comparison of field-contaminated samples of maize analyzed by the method and by HPLC.

In the absence of antibody the sample mixture typically produced a signal of ~90−100 mP. A 1:300 dilution of antibody (3.9 μg/mL final concentration) increased the signal to ~200−225 mP, giving a signal range of 125 mP in which the assays were conducted. The response of the assay to FB_1 in buffer was assessed over the concentration range of 0.002−20 μg/mL (Figure 3). For simplicity in comparing FB_1 contents of standards and samples, the data in Figure 3 for FB_1 in buffer are represented in terms of the equivalence of micrograms per gram in maize rather than micrograms per milliliter. The extraction procedure that was used for maize involved a 5:1 ratio of buffer to maize (v/w); therefore, the extract of a maize sample containing 5 ppm of FB_1 could contain at most 1 μg of FB_1/mL. As such, the range of 0.002−20 μg/mL in buffer corresponded to a range of 0.01−100 μg/g in maize samples. This process provided a mechanism for predicting the best response that could be expected in maize using data collected in buffer and provided a framework for determining the extent to which sample matrix affects parameters of the assay such as sensitivity.

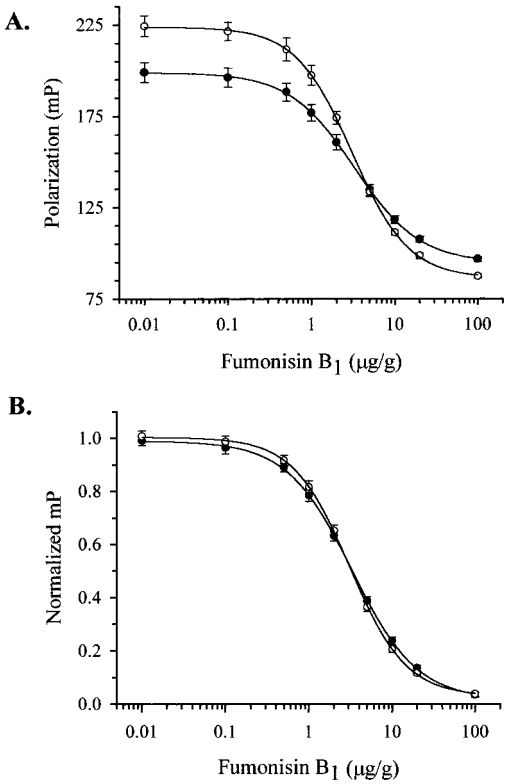

Figure 3. Normalization of fumonisin B_1 standard curves: (A) raw fluorescence polarization data (mP) for FB_1 in PBS (○) and in extracts of spiked maize (●); (B) fluorescence polarization data normalized to scale the mP response to between 0 and 1 as described in the text; response of FB_1 in PBS (○) and in spiked maize (●). For this comparison the data for FB_1 in buffer are expressed in terms of micrograms per gram rather than micrograms per milliliter buffer to account for the 5:1 (v/w) extraction ratio used. Each data point represents the mean of nine assays (three replicate samples with triplicate analyses per sample); error bars represent ±1 standard deviation from the mean.

The effect of maize matrix upon the signal is indicated in Figure 3a. Clearly, both the signal range and the shape of the response curve were affected. In buffer the signal went from 82 ± 1 mP in the absence of antibody to 223 ± 7 mP in the presence of antibody (range of 141 mP). However, in spiked maize the range was 107 mP. Without adjustment of data to accommodate for this discrepancy, it would not be feasible to compare data from unknown samples to a standard curve prepared in PBS. For example, without adjustment of the response a sample giving a signal of 175 mP would be interpreted as having roughly 2 μg/g fumonisin from the standard curve in buffer, whereas the same signal would be interpreted as having roughly 1 μg/g fumonisin from the standard curve in spiked maize (Figure 3a).

The matrix effect from the maize could be controlled by putting both the buffer and maize data sets on the same scale. This was done by mathematically adjusting (normalizing) the data to set the maxima at 1 and the minima at 0. This is analogous to adjusting the data

Table 1. Recovery of FB_1 from Spiked Maize

spiking level (μg/g)	percentage recovery ± standard deviation
0.5	103 ± 13 (n = 9)
1	105 ± 12 (n = 9)
2	99.7 ± 9.6 (n = 9)
5	92.0 ± 8.4 (n = 9)
10	83.7 ± 8.0 (n = 8)
20	78.2 ± 7.0 (n = 8)
average	94.3 ± 13.8 (n = 52)

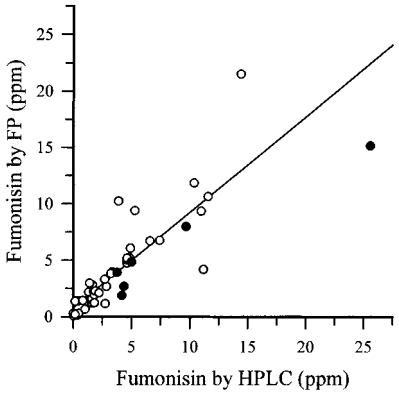

Figure 4. Comparison of HPLC and fluorescence polarization method 2 for determination of fumonisin in contaminated maize. Data are for 48 maize samples analyzed by either HPLC of the OPA derivatized fumonisins (FB_1, FB_2, and FB_3 combined) or total fumonisin as reported by the FP assay. The line is a regression of the data with the equation [fumonisin by FP] = 0.789 + 0.846[fumonisin by HPLC], with a fit of r^2 = 0.880. Solid circles are from six samples that contained more FB_2 than FB_1 by HPLC.

from an ELISA, where the color development is often presented as the percentage of maximum absorbance, that is, color development relative to a toxin-free control. Once expressed on the same adjusted scale, the response of the FB_1 in PBS and the response of FB_1 in spiked maize become quite similar (Figure 3b). The midpoint of the assay is analogous to the IC_{50} in an ELISA assay and was 3.21 μg/g in PBS and 3.38 μg/g in spiked maize, respectively. Recoveries from maize spiked over the range of 0.5−20 μg/g averaged 94.3 ± 13.8% and ranged from 105% at 1 μg/g to 78% at 20 μg/g (Table 1). The recoveries generally decreased with increasing toxin content, suggesting that PBS may be adequate for extracting fumonisins below 20 ppm but may be less efficient above this level.

Comparison of HPLC and FP Method 2 with Field-Inoculated Maize Samples. Because of the presence of matrix effects it was not desirable to directly compare FP signals from samples to those of a standard curve in buffer. However, by representing the data on a scale from 0 to 1 (normalization), the adjusted data from the 48 samples of field-inoculated maize were strongly correlated with the fumonisin determined by HPLC (Figure 4). The linear regression fit of the data (r^2 = 0.880) is similar to that observed with method 1. As with method 1, the discrepancy between FP and HPLC increased along with the fumonisin content. The discrepancy was relatively minor until the fumonisin content became fairly high (≥20 ppm). Interestingly, the recoveries of FB_1 using the PBS extraction appeared to decrease with toxin content (Table 1), yet the FP assay tended to overestimate rather than underestimate the toxin content of highly contaminated samples. We speculate that the presence of a possible interfering component may not be directly proportional to FB_1 content, and perhaps this component increases disproportionately at higher fumonisin levels.

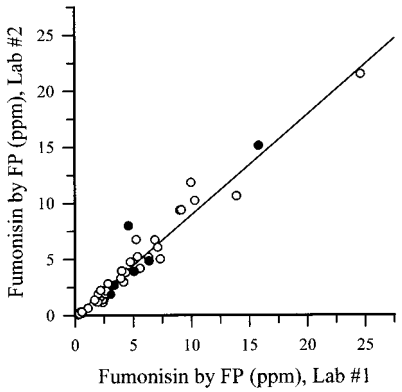

Figure 5. Comparison of two fumonisin FP assays. Laboratory 1 used method 1, with blanking for each sample, and Laboratory 2 used method 2, with a single blank. The methods are illustrated in Figure 1.

Similar to the result seen with method 1, the intercept of the regression line indicated that a sample having no fumonisin by HPLC would, on average, give a response of ~0.8 ppm. As described above for method 1, removing the highly contaminated and high FB_2 samples from the calculation yielded a new regression equation of the form [fumonisin by FP] = 0.211 + 1.13-[fumonisin by HPLC], with a fit of r^2 = 0.72. Unlike with method 1, this treatment improved the intercept (0.2 versus 0.8). However, as with method 1 this treatment worsened the correlation with HPLC (0.72 versus 0.88).

Comparison of Two FP Assays to One Another. The two FP assays, which used different methods of sample preparation, data collection, and data manipulation, in two different laboratories, were nevertheless consistent with each other. The data in Figure 5 are for the 48 field-inoculated maize samples that were analyzed by both FP methods. The good correlation between the two methods (r^2 = 0.977) has several implications. First, it is apparent that the technology is fairly robust, as two different permutations of the technology gave similar results. Second, it is apparent that there are several possibilities for sample handling and data reduction that can be used effectively. This suggests the FP technology can be further tailored to meet a variety of demands. For example, method 1, by using each sample as its own matrix blank, effectively reduced matrix interferences. However, this required inserting each sample into the instrument twice (during blanking and after adding tracer). Method 2 controlled matrix interferences through mathematical correction, the advantage of which is that samples need to be inserted only once. The second method is also useful for gauging matrix effects. A disadvantage of mathematical correction is that two additional samples must be run in each data set to allow correction. Furthermore, with mathematical correction there is the potential for values obtained with a single blanking to be incorrect if the sample background differs substantially from that used to prepare the control standard curve. Both methods tended to overestimate toxin content at lower levels of contamination. The intercept of the comparison of both methods to HPLC indicate a "fumonisin-free" maize sample could be expected to give a result between 0.2 and 1 ppm by FP, depending upon the regression line used for the comparison. Clearly, this issue must be addressed before either FP assay could be used as a

commercial test. Of the two FP methods we report here we preferred method 1 because less data manipulation was required and the assay may be able to tolerate a greater range of sample matrices. Method 2 was valuable for visualizing the impact of maize interferences upon the shape of the standard curve and also showed greater accuracy at lower fumonisin concentrations.

Summary and Conclusions. In addition to yielding results that were correlated with HPLC, the FP assays required little training and were rapid. Following extraction the analysis of each sample took <2 min. Indeed, it took more time to generate the standard curve in preparation for measuring the maize samples than it took to perform the assays. The current software is still under development by the manufacturer, and expanding the data reduction has the potential to further increase the speed of the method. There were benefits, and drawbacks, to both methods: method 1 was better at reducing background fluorescence and did not require data normalization, but method 2 was better at controlling the effects of the matrix upon the antibody and was better for samples containing <8 ppm of fumonisin.

The greatest limitation of the methods was the extraction, which involved shaking the sample with buffer for 1−2 h. Most published ELISA methods use an extraction procedure that involves extended shaking or stirring of the ground maize with solvent or buffer, with extraction times ranging from 30 min to overnight. However, a more rapid extraction method, such as blending for 2−3 min (*39*−*41*), also warrants investigation. Limited data in our laboratories (not shown) suggest it may be possible to incorporate a rapid (5 min) extraction procedure with the FP assay.

Combining a rapid extraction technique with the FP assay would provide a rapid analytical system for fumonisins. This format would appear to be ideally suited to situations requiring samples to be analyzed one at a time (serially) but in a rapid fashion, for example, at grain elevators during harvesting. Although the FP assay can potentially be adapted to a 96-well microtiter plate format, the advantages of such a format over rapid ELISA plates are less obvious.

In conclusion, the fluorescence polarization assays described were useful for screening of maize samples for fumonisin over the range of 0.5−100 μg/g (ppm). The FP technology may therefore have significant potential for screening of maize samples for fumonisins. Many of the existing screening assays for fumonisins are rapid, take <30 min, and are sensitive. The FP assay that we have described is not as sensitive as the best of the fumonisin ELISAs, which can detect nanogram per gram (ppb) levels of these toxins in foods. The sensitivity of the current FP assay, with a limit of detection of 0.5 μg/g, is adequate for screening maize samples, and the simplicity, portability, and ease of use suggest widespread application to mycotoxin analysis should be possible.

SAFETY

The fumonisins have been causally related to several animal diseases and should be handled with appropriate caution. Similarly, maize samples suspected to contain fumonisins should be handled in such a manner as to minimize exposure to contaminated dust during sample collection and grinding.

ACKNOWLEDGMENT

We express appreciation to Dr. Edward Corrigan, Diachemix Corp., for helpful comments and suggestions.

LITERATURE CITED

(1) Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun*. **1988**, 743−745.

(2) Wilson, T. M.; Ross, P. F.; Owens, D. L.; Rice, L. G.; Green, S. A.; Jenkins, S. J.; Nelson, H. A. Experimental reproduction of ELEM. *Mycopathologia* **1992**, *117*, 115−120.

(3) Colvin, B. M.; Harrison, L. R. Fumonisin-induced pulmonary edema and hydrothorax in swine. *Mycopathologia* **1992**, *117*, 79−82.

(4) Haschek, W. M.; Motelin, G.; Ness, D. K.; Harlin, K. S.; Hall, W. F.; Vesonder, R. F.; Peterson, R. E.; Beasley, V. R. Characterization of fumonisin toxicity in orally and intravenously dosed swine. *Mycopathologia* **1992**, *117*, 83−96.

(5) Gelderblom, W. C. A.; Jaskiewicz, K. J.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806−1811.

(6) Marasas, W. F. O. Fumonisins: history, worldwide occurrence and impact. In *Fumonisins in Food*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 1−17.

(7) National Institutes of Health. *NTP technical report on the toxicology and carcinogenesis studies of fumonisin B_1 (CAS Registry No. 116355-83-0) in F344/N rats and B6C3F1 mice*; NTP TR 496. NIH Publication 99-3955; U.S. Department of Health and Human Services: Washington, DC, 1999.

(8) Shephard, G. S. Chromatographic determination of the fumonisin mycotoxins. *J. Chromatogr. A* **1998**, *815*, 31−39.

(9) Plattner, R. D. Detection of fumonisins produced in *Fusarium moniliforme* cultures by HPLC with electrospray MS and evaporative light scattering detectors. *Nat. Toxins* **1995**, *3*, 294−298.

(10) Wilkes, J. G.; Sutherland, J. B.; Churchwell, M. I.; Williams, A. J. Determination of fumonisins B_1, B_2, B_3, and B_4 by high-performance liquid chromatography with evaporative light-scattering detection. *J. Chromatogr. A* **1995**, *695*, 319−323.

(11) Plattner, R. D.; Norred, W. P.; Bacon, C. W.; Voss, K. A.; Peterson, R.; Shackelford, D. D.; Weisleder, D. A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia. *Mycologia* **1990**, *82*, 698−702.

(12) Musser, S. M. Quantitation and identification of fumonisins by liquid chromatography/mass spectrometry. In *Fumonisins in Food*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 65−74.

(13) Maragos, C. M. Analysis of mycotoxins with capillary electrophoresis. *Sem. Food Anal.* **1998**, *3*, 353−373.

(14) Azcona-Olivera, J. I.; Abouzied, M. M.; Plattner, R. D.; Pestka, J. J. Production of monoclonal antibodies to the mycotoxins fumonisins B_1, B_2, and B_3. *J. Agric. Food Chem*. **1992b**, *40*, 531−534.

(15) Fukada, S.; Nagahara, A.; Kikuchi, M.; Kumagai, S. Preparation and characterization of anti-fumonisin monoclonal antibodies. *Biosci., Biotechnol., Biochem*. **1994**, *58*, 756−767.

(16) Pestka, J. J.; Azcona-Olivera, J. I.; Plattner, R. D.; Minervini, F.; Doko, M. B.; Visconti, A. Comparative assessment of fumonisin in grain-based foods by ELISA, GC-MS, and HPLC. *J. Food Prot.* **1994**, *57*, 169−172.

(17) Shelby, R. A.; Rottinghaus, G. E.; Minor, H. C. Comparison of thin-layer chromatography and competitive immunoassay methods for detecting fumonisin on maize. *J. Agric. Food Chem.* **1994**, *42*, 2064−2067.
(18) Usleber, E.; Straka, M.; Terplan, G. Enzyme immunoassay for fumonisin B_1 applied to corn-based food. *J. Agric. Food Chem.* **1994**, *42*, 1392−1396.
(19) Elissalde, M. H.; Kamps-Holtzapple, C.; Beier, R. C.; Plattner, R. D.; Rowe, L. D.; Stanker, L. H. Development of an improved monoclonal antibody-based ELISA for fumonisin B_{1-3} and the use of molecular modeling to explain observed detection limits. *Food Agric. Immunol.* **1995**, *7*, 109−122.
(20) Chu, F. S.; Huang, X.; Maragos, C. M. Production and characterization of anti-idiotype and anti-anti-idiotype antibodies against fumonisin B_1. *J. Agric. Food Chem.* **1995**, *43*, 261−267.
(21) Maragos, C. M.; Plattner, R. D.; Miklasz, S. D. Determination of hydrolyzed fumonisin B_1 (HFB_1) in corn by competitive direct enzyme-linked immunosorbent assay. *Food Addit. Contam.* **1996**, *13*, 105−114.
(22) (a) Yu, F. Y.; Chu, F. S. Production and characterization of antibodies against fumonisin B_1. *J. Food Prot.* **1996**, *59*, 992−997. (b) Yu, F. Y.; Chu, F. S. Production and characterization of monoclonal antibodies against fumonisin B_1. *Food Agric. Immunol.* **1999**, *11*, 297−306.
(23) Kulisek, E. S.; Hazebroek, J. P. Comparison of extraction buffers for the detection of fumonisin B_1 in corn by immunoassay and high performance liquid chromatography. *J. Agric. Food Chem.* **2000**, *48*, 65−69.
(24) Chu, F. S. Immunochemical methods for fumonisins. In *Fumonisins in Food*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 123−133.
(25) Dietrich, R.; Schneider, E.; Usleber, E.; Märtlbauer, E. Use of monoclonal antibodies for the analysis of mycotoxins. *Nat. Toxins* **1995**, *3*, 288−293.
(26) Abouzied, M. M.; Pestka, J. J. Simultaneous screening of fumonisin B_1, aflatoxin B_1, and zearalenone by line immunoblot: a computer-assisted multianalyte assay system. *J. AOAC Int.* **1994**, *77*, 495−500.
(27) Schneider, E.; Usleber, E.; Märtlbauer, E. Rapid detection of fumonisin B_1 in corn-based food by competitive direct dipstick enzyme immunoassay/enzyme-linked immunofiltration assay with integrated negative control reaction. *J. Agric. Food Chem.* **1995**, *43*, 2548−2552.
(28) Maragos, C. M. Detection of the mycotoxin fumonisin B_1 by a combination of immunofluorescence and capillary electrophoresis. *Food Agric. Immunol.* **1997**, *9*, 147−157.
(29) Thompson, V. S.; Maragos, C. M. Fiber-optic immunosensor for the detection of fumonisin B_1. *J. Agric. Food Chem.* **1996**, *44*, 1041−1046.
(30) Dandliker, W. B.; Feigen, G. A. Quantification of the antigen−antibody reaction by the polarization of fluorescence. *Biochem. Biophys. Res. Commun.* **1961**, *5*, 299−304.
(31) Haber, E.; Bennett, J. C. Polarization of fluorescence as a measure of antigen−antibody interaction. *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 1935−1942.
(32) Checovich, W. J.; Bolger, R. E.; Burke, T. Fluorescence polarization—a new tool for cell and molecular biology. *Nature* **1995**, *375*, 254−256.
(33) Dandliker, W. B.; Kelly, R. J.; Dandliker, J.; Farquhar, J.; Levin, J. Fluorescence polarization immunoassay. Theory and experimental method. *Immunochemistry* **1973**, *10*, 219−227.
(34) Jolley, M. E. Fluorescence polarization immunoassay for the determination of therapeutic drug levels in human plasma. *J. Anal. Toxicol.* **1981**, *5*, 236−240.
(35) Nasir, M. S.; Jolley, M. E. Fluorescence polarization: an analytical tool for immunoassay and drug discovery. *Comb. Chem. High Throughput Screening* **1999**, *2*, 177−190.
(36) Nakayama, H.; Kitawaki, F.; Miyazaki, J. Fluorescence polarization method at multiple wavelengths for determination of multiple analytes. Eur. Patent Appl. 2000.
(37) Maragos, C. M.; Bennett, G. A.; Richard, J. L. Affinity column cleanup for the analysis of fumonisins and their hydrolysis products in corn. *Food Agric. Immunol.* **1997**, *9*, 3−12.
(38) Shephard, G. S.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **1990**, *13*, 2077−2087.
(39) Ware, G. M.; Umrigar, P. P.; Carman, A. S., Jr.; Kuan, S. S. Evaluation of fumonitest immunoaffinity columns. *Anal. Lett.* **1994**, *27*, 693−715.
(40) Trucksess, M. W.; Stack, M. E.; Allen, S.; Barrion, N. Immunoaffinity column coupled with liquid chromatography for determination of fumonisin B^1 in canned and frozen sweet corn. *J. AOAC Int.* **1995**, *78*, 705−710.
(41) Sydenham, E. W.; Stockenström, S.; Thiel, P. G.; Rheeder, J. P.; Doko, M. B.; Bird, C.; Miller, B. M. Polyclonal antibody-based ELISA and HPLC methods for the determination of fumonisins in corn: a comparative study. *J. Food Prot.* **1996**, *59*, 893−897.

Received for review August 28, 2000. Revised manuscript received November 20, 2000. Accepted November 20, 2000. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the products, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

JF0010782