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A comparative study of solvent extraction efficiency and the performance of immunoaffinity and solid phase columns on the determination of aflatoxin B_1

N. Bradburn, R. D. Coker

Natural Resources Institute, Central Avenue, Chatham, Maritime, Chatham, Kent ME4 4TB, UK

& G. Blunden

School of Pharmacy and Biomedical Sciences, University of Portsmouth, Park Building, King Henry I Street, Portsmouth, Hants, P01 2DZ, UK

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The efficiency of two different immunoaffinity columns and a phenyl bonded-phase column were compared during the extraction, clean-up and quantification of aflatoxin B_1 from sorghum and maize. Fluorodensitometry of high performance thin-layer chromatograms was used for quantification of aflatoxin B_1.

Maize had a simple matrix, and comparable precisions and accuracies were obtained for each of the methods.

The sorghum matrix was complex and the bonded-phase procedure was the most accurate and precise method. There was evidence to suggest that the lower aflatoxin B_1 recovery from sorghum by immunoaffinity columns is a solvent extraction problem.

Better aflatoxin B_1 recoveries were obtained from naturally contaminated sorghum and maize when acetonitrile was replaced with acetone as the extraction solvent.

INTRODUCTION

Aflatoxins are a group of highly toxic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus*. They exhibit carcinogenic, teratogenic and mutagenic properties and have been isolated from a wide variety of agricultural products. Currently, 56 countries have either imposed or are considering the introduction of regulations controlling the amount of aflatoxin entering the food chain (Van Egmond, 1989). To obtain reliable values of total aflatoxin depends on suitable statistically based sampling plans in combination with rapid, simple, reproducible, sensitive and cost-effective assay procedures.

Traditional analytical methods for the determination of aflatoxin employ column chromatography, liquid–liquid partition or chemical adsorption methods for removing interfering compounds. Subsequent quantification is often performed by either thin-layer chromatography (TLC) or high-performance liquid chromatography separation and suitable fluorimetric quantification. As well as being time consuming and costly, these approved clean-up procedures (EEC, 1976; AOAC, 1984) are only partially effective and do not remove all the substances with similar chromatographic and fluorescent properties to those of aflatoxins in extracts of some commodities. Quantification can then result in false positive or, more dangerously, false negative results.

Solid-phase extraction and immunoaffinity procedures have greatly simplified the sample clean-up stage and provided high purity extracts that can be used with modern sensitive detection methods (Bradburn *et al.*, 1989; Bradburn *et al.*, 1990*b*; Carvajal *et al.*, 1990; Patey *et al.*, 1991; Trucksess *et al.*, 1991). The procedures use relatively small volumes of solvent and can be used with automated sample handling devices, which reduce analysis time and increase throughput.

The Aflatest immunoaffinity column, coupled with either solution fluorimetry or liquid chromatography with postcolumn derivatisation, has been adopted as

the Official First Action Method by the Association of Official Analytical Chemists (AOAC) for the determination of aflatoxin in corn, raw peanuts and peanut butter (Trucksess *et al.*, 1991) at total aflatoxin concentrations of ≥10 μg/kg. Easi-Extract immunoaffinity columns have also been assessed in a collaborative trial, for use with peanut butters, but as yet have not been adopted as an Official First Action AOAC Method (Patey *et al.*, 1991). The United States Department of Agriculture's Federal Grain Inspection Service (USDA-FGIS) has approved the Aflatest and Easi-Extract test kits as alternative methods for the screening of maize samples (Emnett, 1989).

Phenyl bonded-phase cartridges have been successfully applied to the analysis of aflatoxin in cottonseed (Bradburn *et al.*, 1989) and maize (Bradburn *et al.*, 1990*a*) using the First Action AOAC Contamination Bureau (CB) method for comparison. Easi-Extract immunoaffinity columns (Carvajal *et al.*, 1990) have also been compared with the AOAC CB method for raw, ground unskinned peanuts. Both the bonded-phase and immunoaffinity methods gave better aflatoxin B_1 recoveries and comparable precision when compared to the standard AOAC CB method.

It has long been recognised that the choice of solvent for extraction of aflatoxin from foods and feeds is strongly matrix dependent. One solvent system may provide good extraction with one commodity, but prove less effective with another. Chloroform, acetonitrile, acetone and particularly methanol are commonly used to extract aflatoxins. Although it is desirable to increase aflatoxin extraction efficiency, extraction of other compounds that interfere with the analysis should be kept to a minimum. It has been found (Bradburn *et al.*, 1990*b*) that 80% aqueous acetone extracts more aflatoxin from maize than do other solvent systems.

This paper compares the efficiency of phenyl bonded-phase columns and two types of immunoaffinity columns during the extraction and clean-up of aflatoxin B_1 from sorghum and maize. The effect of the initial extraction solvent on the recovery of aflatoxin from each immunoaffinity method is also investigated.

MATERIALS AND METHODS

Apparatus

A cyclone mill (Cyclotec 1093, Tecator AB, Hüganäs, Sweden) with a 0·5 mm screen and a cutter mill with a 1 mm screen (Apex 116A, Apex Construction Ltd, Kent, UK) were used for grinding sorghum and maize, respectively. Flameproof 1 litre Waring blenders (Dynamics Corporation of America, New Hartford, CT, USA) were used for slurry preparation and organic solvent–water extractions.

The clean-up apparatus (Jones Chromatography, Mid Glamorgan, UK) consisted of a vacuum manifold (Vacelut A16000) with disposable 500 mg phenyl (PH) bonded-phase columns (PH: 608 303) coupled with 4 ml (6 004 000) and 75 ml (607 500) reservoirs, and the corresponding 20 μm porous frits (640 410 and 647 510, respectively). Connections were made using adapters (636 001) and luer stopcocks (A 16078). This apparatus was also compatible with Aflaprep affinity columns (Rhône-Poulenc Diagnostics, Glasgow, UK). Total aflatoxin Easi-Extract affinity columns (Biocode, York, UK) can also be used with the apparatus by substituting different adaptors (700/180/711: Orme Scientific, Manchester, UK). Both types of immunoaffinity columns were stored at 5°C, and allowed to reach room temperature before use.

A sample concentrator (DM-Block DB-3: Tecam, UK) was used for evaporation of the chloroform and acetonitrile extracts prior to quantification. A Perkin-Elmer Lambda 3 ultraviolet (UV)–visible spectrophotometer was used to determine the concentration of the aflatoxin standards and spiking solutions. Aluminium backed high-performance TLC (HPTLC) plates, without fluorescent indicator (Merck 5547), were spotted using an automated TLC sampler (27 200: Camag, Switzerland). A conventional universal Chromatank (SAB 2842, Shandon, UK) and continuous linear development TLC tank (Bradburn *et al.*, 1990*a*) were employed during the chromatographic development of the plates. A TLC scanner II (76610: Camag) and TLC integrator SP 4270 (76650: Camag) controlled by a personal computer with Link-up software (Quadrant Scientific, UK) were employed for the densitometry measurements.

Samples

Naturally occurring aflatoxin B_1 contaminated and uncontaminated samples of both Indian sorghum and Pakistani maize were used throughout this investigation. Whatman No. 1 filter papers were used to filter all slurry extracts.

Reagents

Phosphate-buffered saline (PBS) was prepared by dissolving 0·20 g of potassium chloride, 0·02 g of disodium hydrogen phosphate, 1·16 g of disodium hydrogen phosphate and 8·00 g of sodium chloride in *c.* 900 ml of deionised water. After adjusting the pH to 7·4 the solution was made up to 1000 ml.

Lead acetate solution (20%, w/v) was prepared by dissolving 100 g of lead acetate trihydrate in *c.* 300 ml of water with warming. Acetic acid (1·5 ml) was added to this and the volume was made up to 500 ml. All chemicals were AnalaR grade (BDH Chemicals, Poole, UK).

Aflatoxin standards

Crystalline aflatoxin B_1 standard (Sigma Chemical, UK) was dissolved in benzene–acetonitrile (98: 2, v/v) to give concentrations of *c.* 10 μg/ml. The absolute

concentrations were determined by UV absorbance at 366 nm. These stock solutions were diluted with benzene–acetonitrile (98:2) to give standard solutions with aflatoxin (AF) concentrations of 1·0 μg/ml (AFB_1, AFG_1) and 0·5 μg/ml (AFB_2, AFG_2). They were stored at −20°C prior to use.

Procedures

Preparation of extracts
Slurries (50 g) were formed by blending naturally contaminated, comminuted samples at high speed for 3 min; using meal–water ratios of 1·5:2 and 1:1·25 (w/v) for sorghum and maize, respectively. Two acetone–water extracts (80:20, v/v) of sorghum and maize were prepared by blending 50·0 and 48·6 g, respectively, of each slurry with 111 ml of acetone, and filtering. Two acetonitrile–water extracts (75:25, v/v) of sorghum and maize were also prepared by blending 66·6 and 64·8 g, respectively, of each slurry with 111 ml of acetonitrile, and filtering. This was repeated for the uncontaminated commodities.

Phenyl bonded-phase cartridge clean-up
Precleaned diatomaceous earth (1 g) was added to a reservoir (75 ml), fitted with a porous frit, and attached to a phenyl column previously conditioned by the addition of methanol (10 ml) and then water (10 ml). The column was not allowed to run dry. Water–methanol–acetic acid (92·3:6·7:1·0; 30 ml), acetone extract (5 ml) and lead acetate (3 ml) were added to the reservoir and, rapidly, a further quantity of water–methanol–acetic acid (30 ml) was introduced into the reservoir to ensure that the contents were adequately mixed. Negative pressure, supplied by a water pump, was applied to the cartridge so that the mixture passed through at a flow rate of 10 ml/min. Finally, water (10 ml) was drawn through the column to remove extraneous materials.

The reservoir was removed and the column was dried by passing air through it for 5 min. A reservoir (4 ml) fitted with a porous frit and containing granular anhydrous sodium sulphate (3 g) was connected to the base of the PH column and the aflatoxin B_1 was eluted from the sorbent with chloroform (7 ml; 1 ml/min). The eluate containing the aflatoxins was evaporated to dryness at 50°C under a gentle stream of nitrogen in a sample concentrator.

Immunoaffinity column clean-up
Aliquots (5 ml) of the aqueous acetonitrile extract were diluted to 120 ml with PBS in the case of the Easi-Extract column, and to 60 ml with water for the Aflaprep column. These were added to a reservoir (75 ml) and drawn through the respective immunoaffinity columns, which had been previously washed with 10 ml of PBS, at a flow rate of 1 ml/min.

Elution of aflatoxin from the immunoaffinity columns
The columns were washed with two 10 ml aliquots of water and allowed to dry by passing air through them for 2 min. The dried columns were connected to the tops of 4 ml reservoirs fitted with 20 μm frits and containing anhydrous sodium sulphate (1 g). The aflatoxins were eluted from the columns by passing acetonitrile through the columns at the slowest possible rate (0·3 ml/min). The eluates were evaporated to dryness under a stream of nitrogen in a sample concentrator.

Comparison of the clean-up methods
Each clean-up method was applied to five replicate aliquots of sorghum and maize extracts; aflatoxin B_1 was determined by bidirectional HPTLC.

Evaluation of solvent extraction
In order to ascertain the efficiency of aqueous acetone extraction of aflatoxin B_1, the Aflaprep (A.Std) and Easi-Extract (E.Std) immunoaffinity methods described above were modified (A.Mod and E.Mod) by using phenyl bonded-phase columns to effect a change in solvent from aqueous acetone to acetonitrile.

Fifteen aliquots of aqueous acetone extracts of sorghum and maize were cleaned-up using the phenyl bonded-phase method. Two batches of five extracts for each commodity were selected at random, reconstituted in acetonitrile, and subjected to the immunoaffinity procedures. All 30 final extracts were quantified using bidirectional HPTLC.

Confirmation of extraction efficiency
In order to assess the extraction efficiency of the aqueous solvent systems, five aliquots of each filtrate were subjected to the phenyl bonded-phase (PH) method. Column retention was monitored by collecting the eluates. These were reduced to dryness on a rotary evaporator, and quantified alongside the chloroform extracts, using bidirectional HPTLC.

Sample clean-up comparison
The uncontaminated extracts were subjected to each method and quantified alongside the contaminated extracts.

Quantification using bidirectional HPTLC
HPTLC plates (20 cm × 20 cm) were cut in half and immersed in methanol for 1 h in order to remove atmospheric contaminants deposited on the plate during storage. The plates were dried at 100°C in a fan-assisted oven (5 min) and stored in a desiccator until required.

The following procedures were performed under darkened conditions. Clean, dried sample extracts were dissolved in 300 μl of benzene–acetonitrile (98:2). Aliquots (5 μl) of this extract and aliquots (1 μl) of mixed aflatoxin standard solution were applied, using an autosampler, as a row of spots at 5 mm intervals 3 cm from the top edge of a HPTLC plate.

A strip of silica gel (3 mm wide) was removed from the edges of the plate, parallel to the direction of development, in order to eliminate edge effects. A further clean-up step was performed by developing the plates

in anhydrous diethyl ether (20 ml) for 17 min in a continuous linear development chamber (Bradburn *et al.*, 1990*b*). The plate was dried (3 min) in a darkened chamber under a stream of nitrogen, and the top portion (2 cm) of the plate, containing sample interferences, was removed with a sharp knife. The plates were rotated through 180° and the aflatoxins were separated by two 20 min developments in 20 ml of chloroform–xylene–acetone (6:3:1). The solvent was evaporated from the plates, in a stream of nitrogen, between developments. After the second development, the plates were dried (1 min) in a fan-assisted oven at 100°C.

Fluorodensitometric quantification at 366 nm was performed in the fluorescence reflection mode, using a high pressure mercury lamp. A scan speed of 0·5 mm/s, an optical slit width of 4 mm × 0·2 mm and a single level of 1·0 ng of aflatoxin B_1 for external standard calibration were used for scanning. Only the areas containing aflatoxin B_1 were scanned (R_F value 0·43). An illustration of such a chromatogram has been published by Tomlins *et al.* (1989).

Calculation of effective weight

The effective weights of the extracts were 0·750 g for sorghum and 0·801 g for maize. They were calculated using the following equation:

$$\text{Effective weight} = \frac{V_{ex} \times M_{smp}}{M_{sl} - M_{smp} + V_{sol}}$$

where V_{ex} = volume of extract used in analysis, M_{smp} = mass of sample of slurry, M_{sl} = mass of slurry, and V_{sol} = volume of organic solvent used for extraction.

Statistics

Comparison of recoveries and precisions were performed using a significance level of 5% (Miller & Miller, 1984). If, on application of an *F*-test, there was no significant difference between the standard deviations of the two methods (S_1 and S_2) then a 'pooled standard deviation' (S_p) was calculated (Miller & Miller, 1984):

$$S_p^2 = \{ (n_1-1)S_1^2 + (n_2-1)S_2^2) / (n_1 + n_2-2)$$

A *t*-test was then used to determine a significant difference between method means ($\bar{x}_1$ and $\bar{x}_2$):

$$t = (\bar{x}_1 - \bar{x}_2) / s_p \sqrt{1/n_1+1/n_2}$$

where t has (n_1+n_2-2) degrees of freedom (df). If a significant difference between the standard deviations was detected, then $|t|$ was calculated directly from the two individual standard deviations:

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{s_1^2/n_1+s_2^2/n_2}$$

and the number of degrees of freedom was given by

$$\text{df} = \left\{ \frac{(s^2/n_1+s^2/n_2)^2}{\frac{(s^2/n_1)^2}{n_1+1} + \frac{(s^2/n_2)^2}{n_2+1}} \right\} - 2$$

RESULTS AND DISCUSSION

The extracts produced by the immunoaffinity methods were the cleanest and would not normally require the extra clean-up, necessary for the bonded-phase extracts, provided by bidirectional HPTLC. Comparison of the contaminated and uncontaminated extracts for each method showed that there were no fluorescent interferences on the HPTLC plate. Replicate aflatoxin B_1 recoveries for sorghum and maize extracts by the five methods are shown in Table 1. Aflatoxin B_2 was found in only trace amounts in some samples and was not estimated; aflatoxins G_1 and G_2 were never detected.

Comparison of the mean recoveries and precisions of the methods for aflatoxin B_1

Sorghum

When the mean recoveries of each method were ranked the 95% confidence intervals (Table 2) for the A.Mod, E.Mod, A.Std and E.Std methods, overlapped, indicating similar accuracies. As the confidence intervals for the highest ranked PH method did not overlap those of any other method it was considered to be the most accurate.

The precisions of the methods were dissimilar, with coefficients of variation (CVs) of 1·7 to 12·5%. Application of a two-tailed *F*-test showed significant differences between the precisions of the PH method and the standard and modified immunological methods. The observed *F* ratios (F_{obs}) of the variances of the methods ($F_{PH\text{-}A.Srd}$ = 21·906; $F_{PH\text{-}A.Mod}$ = 19·217; $F_{PH\text{-}E.Std}$ = 10·921; $F_{PH\text{-}E.Mod}$ = 35·041) were greater than the critical two-sided *F* statistic ($F_{0.975}(4,4)$ = 9·6), showing that the methods were not of comparable precision. In order to determine significant differences between the experimental methods, $|t|$ values were obtained directly from the two individual standard deviations ($|t|$ PH-A.Std = 7·30; $|t|$ PH-A.Mod = 5·44; $|t|$ PH-E.Std = 12·86; $|t|$ PH-E.Mod = 4·35). As all these experimental $|t|$ values were above the respective critical $|t|$ values ($|t|$ 0·05(4) = 2·78, $|t|$ 0·05(5) = 2·57) the null hypothesis, that the means of the methods were the same, was rejected.

Improvements, with respect to the standard procedures, in aflatoxin B_1 retention of 10·8 and 20·9% were obtained with the A.Mod and E.Mod methods, respectively. Comparison of the F_{obs} ratios ($F_{A.Std\text{-}A.Mod}$ = 1·14; $F_{E.Std\text{-}E.Mod}$ = 3·21) shows that they have similar precisions. The 'pooled standard deviation' method was used to determine any significant differences between the means. As the experimental $|t|_{A.Std\text{-}A.Mod}$ values of 1·64 is less than the critical $|t|$ statistic of 2·31 the null hypotheses, that the means of the two methods are the same, cannot be rejected. For the Easi-Extract column, where $|t|_{E.Std\text{-}E.Mod}$ = 2·70 is greater than the critical $|t|$ statistic, the null hypothesis could be rejected and it could be concluded that the mean for E.Mod is significantly higher than that for E.Std.

Maize

The 95% confidence intervals of the mean recoveries for the PH, A.Mod and E.Mod methods overlap

Table 1. Concentrations of aflatoxin B_1 found in replicate samples of sorghum and maize when analysed after the use of: a phenyl bonded-phase column clean-up method (PH); standard (A.Std) and modified (A.Mod) methods using Aflaprep immunoaffinity column clean-up; standard (E. Std) and modified (E.Mod) methods using Easi-Extract immunoaffinity column clean-up

Commodity	Aliquot number	Aflatoxin B_1 concentration (μg/kg)				
		PH	A.Std	A.Mod	E.Std	E.Mod
Sorghum	1	106·8	78·2	86·0	77·2	73·7
	2	111·4	89·8	80·2	81·6	98·1
	3	109·8	71·4	96·0	69·2	79·7
	4	109·8	74·5	85·5	69·2	91·2
	5	111·3	90·1	99·9	67·5	97·9
	Mean value ($\bar{x}$)	109·8	80·8	89·5	72·9	88·1
	Standard deviation	1·9	8·7	8·1	6·1	11·0
	S^2	3·5	75·6	66·3	37·7	120·9
	%CV	1·7	10·8	9·1	8·4	12·5
Maize	1	100·4	70·1	105·1	80·1	92·3
	2	96·7	67·4	100·0	77·8	89·9
	3	92·0	79·9	101·9	75·0	98·3
	4	94·9	69·6	95·6	66·7	85·2
	5	100·4	82·0	98·6	79·5	80·0
	Mean value ($\bar{x}$)	96·9	73·8	100·2	75·8	89·1
	Standard deviation	3·6	6·7	3·6	5·5	7·0
	S^2	13·1	44·2	12·7	29·9	48·4
	%CV	3·7	9·0	3·0	7·2	7·8

(Table 2), suggesting similar accuracies. This was also found to be the case for the A.Std, E.Std and E.Mod methods.

The precisions of the methods were similar, with CVs of 3·6 to 7·8%. No significant differences between the precisions of the methods could be found by applying a two-tailed F-test as the F_{obs} ratio of the variances ($F_{PH\text{-}A.Std}$ = 3·363; $F_{PH\text{-}A,Mod}$ = 1·037; $F_{PH\text{-}E.Std}$ = 2·277; $F_{PH\text{-}E.Mod}$ = 3·683; $F_{A.Std\text{-}A.Mod}$ = 3·489; $F_{M.Std\text{-}E.Mod}$ = 1·618) were less than the critical two-sided F statistic ($F_{0.975}(4,4)$ = 9·6)). Thus, the 'pooled standard deviation' method was used to determine any significant differences between the means. When the PH method was compared with the standard immunological procedures, the experimental $|t|$ values $|t|_{PH\text{-}A.Srd}$ = 6·82; $|t|_{PH\text{-}E.Std}$ = 7·17) were greater than the critical $|t|$ statistic of 2·31, showing that the mean value for aflatoxin B_1 is significantly greater with the PH method. The A.Mod and E.Mod methods show a 32·2 and 17·6% improvement, compared to the standard methods, in the recovery of aflatoxin B_1, respectively. When compared with the PH method, both experimental $|t|$ values $|t|_{PH\text{-}A.Mod}$ = 1·48; $|t|_{PH\text{-}E.Mod}$ = 2·21) were less than the critical $|t|$ statistic of 2·31 and the null hypothesis, that the methods give the same mean, could not be rejected. The fact that modifying the immunological methods shows a significant increase in the mean aflatoxin B_1 value is reflected by the experimental $|t|$ ratios of the standard and

Table 2. The 95% confidence intervals for aflatoxin B_1 contents of sorghum and maize when analysed after the use of: a phenyl bonded-phase column clean-up method (PH); standard (A.Std) and modified (A.Mod) methods using Aflaprep immunoaffinity column clean-up; standard (E.Std) and modified (E.Mod) methods using Easi-Extract immunoaffinity column clean-up

Analytical method	Sorghum			Maize		
	Mean aflatoxin B_1 content (μg/kg)	UCL[a]	LCL[b]	Mean aflatoxin B_1 content (μg/kg)	UCL[a]	LCL[b]
PH	109·8	112·1	107·5	96·9	101·4	92·4
A.Std	80·8	91·6	70·0	73·8	82·1	65·5
A.Mod	89·5	99·6	79·4	100·2	110·1	90·3
E.Std	72·9	80·5	65·3	75·8	82·6	69·0
E.Mod	88·1	101·8	74·4	89·1	97·8	80·5

[a]UCL = upper 95% confidence limit = mean value ($\bar{x}$) + $\frac{2{\cdot}78 \times \text{standard deviation}}{\sqrt{\text{number of replicates (5)}}}$.

[b]LCL = upper 95% confidence limit = mean value ($\bar{x}$) − $\frac{2{\cdot}78 \times \text{standard deviation}}{\sqrt{\text{number of replicates (5)}}}$.

Table 3. Comparison of the concentrations of aflatoxin B_1 found in replicate samples of aqueous acetone and acetonitrile extracts of sorghum and maize when analysed after the use of a phenyl bonded-phase column clean-up method

Extraction solvent	Aflatoxin B_1 concentration (μg/kg)	
	Sorghum	Maize
Acetone	84·2	120·0
	80·9	118·2
	82·2	118·0
	81·4	119·7
	83·0	122·5
Mean value ($\bar{x}$)	82·3	119·7
Standard deviation	1·3	1·8
S^2	1·7	3·3
%CV	1·6	1·5
Acetonitrile	70·5	105·7
	70·2	99·9
	68·3	100·1
	68·0	104·7
	67·2	103·3
Mean value ($\bar{x}$)	68·8	102·7
Standard deviation	1·4	2·6
S^2	2·0	7·0
%CV	2·1	2·6

modified immunological methods ($|t|_{\text{A.Std-A.Mod}} = 7{\cdot}83$; $|t|_{\text{E.Std-E.Mod}} = 3{\cdot}36$), which are higher than the critical $|t|$ value of 2·31.

Confirmation of extraction efficiency

Replicate aflatoxin B_1 recoveries from aqueous acetone and acetonitrile extracts of sorghum and maize, using the PH method, are shown in Table 3.

No aflatoxin B_1 was found in the PH column eluates as it was all retained. This allowed the extraction efficiency of aqueous acetone (AC) and acetonitrile (AN) to be compared. The precision of the method for each extraction solvent and commodity was similar, with CVs of 1·5–2·6%. Application of a two-tailed F-test showed no significant difference between the precisions of the method for different aqueous extraction solvents. The F_{obs} ratio of the variances for sorghum and maize, were $F_{\text{AC-AN}} = 1{\cdot}207$ and $F_{\text{AC-AN}} = 2{\cdot}138$, respectively. These were less than the critical two-sided F-statistic ($F_{0{\cdot}975}(4,4) = 9{\cdot}6$), showing that the PH method gave comparable precisions for both extraction solvents. The PH method retained 19·6 and 16·6% more aflatoxin B_1 from aqueous acetone extracts of sorghum and maize respectively. The $|t|$ values were calculated to determine significant differences in mean recovery, using the pooled standard deviation method. The null hypotheses, that the means of the methods were the same, was rejected as the experimental $|t|$ values of $|t|_{\text{ACPH-ANPH}} = 35{\cdot}89$ and $|t|_{\text{ACPH-ANPH}} = 106{\cdot}85$ sorghum and maize, respectively, far exceeded the critical $|t|$ value of 2·32. It would appear that acetone extracts significantly more aflatoxin B_1 than acetonitrile under the same analytical conditions.

CONCLUSIONS

The PH method was more accurate and precise than any of the immunoaffinity procedures for the determination of aflatoxin B_1 in extracts of sorghum. The modified Easi-Extract method was equally as precise, and more accurate than the standard procedure. In contrast, the modified Aflaprep method showed no such statistically significant increase in accuracy, but was equally as precise.

In the case of maize, a significant difference between the precisions of all the methods was found; the PH method was also more accurate than the standard immunological procedures. The modified immunological methods were more accurate than the standard immunoaffinity and PH methods.

Direct comparison of the solvent extraction efficiency, using the PH method, confirmed that the use of acetone resulted in statistically significant increases in extracted aflatoxin B_1 compared to when acetonitrile was used.

For the commodities investigated in this study, the PH method is preferable to the standard immunological procedures. However, it has been demonstrated that the difference in accuracy between the columns is a result of solvent extraction and not column retentiveness. Although not as important as considerations of accuracy and precision, the high cost of immunoaffinity columns is a disadvantage and is an important consideration in Third World countries.

It is known that some sorghum varieties cannot be assayed using the current PH method due to residual interfering compounds, but, for these varieties, a florisil column clean-up method has been successfully used (Jewers *et al.*, 1989). Further investigations are underway to exploit the superior clean-up of immunoaffinity columns.

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