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Note

Comparison of the EASI-EXTRACT immunoaffinity concentration procedure with the AOAC CB method for the extraction and quantitation of aflatoxin B_1 in raw ground unskinned peanuts

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Aflatoxins are secondary metabolites of the moulds *Aspergillus flavus* and *parasiticus* from the chemical group difuranocoumarins. They are potent carcinogens, teratogens, mutagens and toxins and pose a severe hazard to animal and human health[1–6]. The most potent of the four naturally occurring aflatoxins is aflatoxin B_1 (AFB_1). Aflatoxins have been found as contaminants of numerous crops including peanuts, cereals, figs, maize etc.

Official first action AOAC methods have been prepared for the analysis of aflatoxins in peanuts, one of the most commonly contaminated crops. The CB Method I[7] is recommended for analysing aflatoxins in raw and processed peanut products but it is our experience that for routine testing most laboratories pursue some variant of this procedure. Hence, it is often not possible to establish if a particular aflatoxin level found in food is an accurate reflection of the true value. The CB method is time consuming, uses large volumes of solvent and often is affected by interfering substances during the final thin-layer chromatography (TLC) analysis.

To circumvent these problems antibody methods are now beginning to be routinely used for aflatoxin analysis because of their exquisite sensitivity and specificity[8–13]. We have found, that for complex food matrices, enzyme-linked immunoad-

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sorbent assay (ELISA) methods suffer from the disadvantage of non-specific inhibition leading to false positives as well as an inability to quantitate accurately each of the four naturally occurring aflatoxins. It is for these reasons that we have examined a commercial immunoaffinity column for the routine assay of aflatoxins and compared this with the AOAC CB method[7]. We present the results of this comparison here.

EXPERIMENTAL

Materials

Commercial immunoaffinity columns were purchased (EASI-EXTRACT® columns for total quantitative aflatoxin analysis — TD110, Oxoid Ltd., Basingstoke, U.K., manufactured and developed by Biocode Ltd., Heslington, U.K.; EASI-EXTRACT is a trademark of Biocode). AFB_1 and Celite were obtained from Sigma (Poole, U.K.) and silica gel 60 extra pure mesh 70–230 and DC Alufolien Kieselgel 60 Merck TLC plates from BDH (Poole, U.K.). All other chemicals were of laboratory reagent grade.

Spiking of ground peanuts with aflatoxin B_1

A standard solution of AFB_1 at a concentration of 10 μg/ml in dimethyl sulphoxide (DMSO) was prepared using UV spectrophotometry. This was used to prepare 100 and 500 ng/ml solutions by dilution in chloroform for spiking ground peanut samples. The latter were prepared by grinding raw, skinned peanuts obtained from a local health food store in a coffee grinder so that they would pass through a medium sieve. An amount of 480 g of the ground peanuts were aliquoted as detailed in Fig. 1.

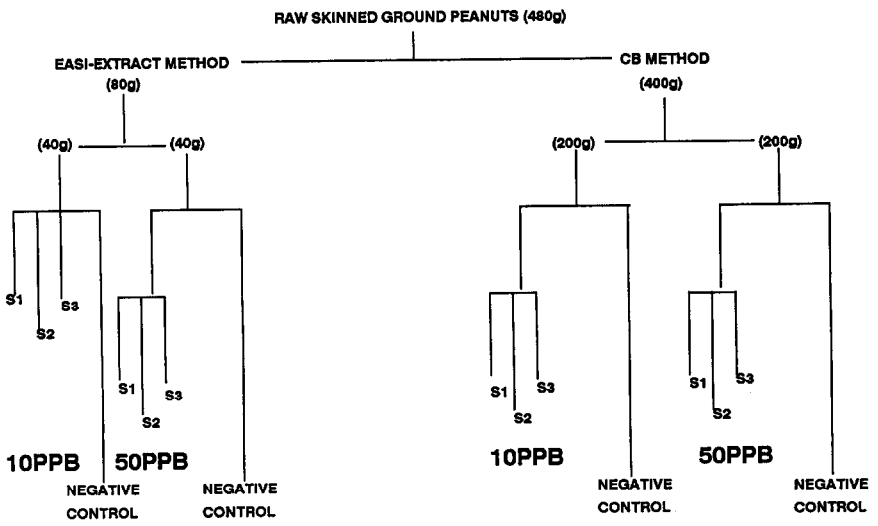

Fig. 1. Spiking and extraction scheme used in this study. S1, S2, S3 and the negative control were equal weight aliquots that were extracted as separate samples, *i.e.* for the EASI-EXTRACT procedure, 10 g and the AOAC method, 50 g.

Samples were spiked with AFB_1 at the levels indicated and the mycotoxin recovered using the EASI-EXTRACT or CB methods (see below).

Recovery of aflatoxin B_1 from spiked ground peanuts

EASI-EXTRACT immunoaffinity chromatography. An amount of 10 g of spiked ground peanut sample was blended using a Ultra-Turrax homogeniser (Janke and Kunkel from Sartorius, Belmont, U.K.) for 2 min in 20 ml acetonitrile–water (3:2, v/v) and then centrifuged at 860 *g* for 10 min. The supernatant was recovered and 4 ml taken and diluted with 92 ml of phosphate-buffered saline (PBS; potassium chloride 0.2 g, potassium dihydrogen orthophosphate 0.2 g, disodium hydrogen orthophosphate 1.16 g, sodium chloride 8 g, made up to 1 l with distilled water, pH 7.4). This was passed through the EASI-EXTRACT column by means of a 50-ml disposable syringe such that the liquid emerged from the nozzle in a steady stream in which individual drops were visible. The column was washed with 10 ml PBS to remove any contaminating matter and the bound AFB_1 recovered by eluting with 2 ml acetonitrile. The solvent was removed by blowing down with nitrogen and the resulting AFB_1 dissolved as described below prior to TLC.

AOAC CB method. An amount of 50 g of spiked peanut sample was placed in a flask with chloroform (250 ml), distilled water (25 ml) and Celite (25 g) and the flask shaken on a wrist action shaker for 30 min. The resulting extract was filtered through Whatman 41 filter paper and processed as per the official AOAC first action CB method[7]. The final chloroform solution was evaporated under nitrogen prior to TLC analysis.

Thin-layer chromatography

The dried down AFB_1 was redissolved in 200 μl chloroform for the EASI-EXTRACT samples and 200 μl of benzene–acetonitrile (98:2, v/v) for the CB recovered samples. Aliquots were spotted onto precoated aluminium backed silica gel plates which had been activated for 120 min at 100°C prior to sample application. Samples of standard AFB_1 solutions were spotted onto the same plates in order that comparisons could be made in fluorescence intensity between these standards and the recovered spiked samples. The plates were developed in acetone–chloroform (1:9, v/v) and air-dried before visualisation of the spots under a UV lamp (365 nm). Three spots, applied by microsyringe, from each spiked sample, negative controls and external standards (1, 2.5, 5, 7.5 and 10 ng applied to the plate) were used to quantitate recovery. Where the sample spot fluorescence fell between standards the mean of the two standards was used for calculation purposes. Where the match could not easily be made the chromatography run was repeated using more appropriate concentrations of either standard or sample. All procedures were performed by the same analyst. Quantitation was confirmed by a second person who was unaware of the sample or standard codes.

RESULTS AND DISCUSSION

As far as we are aware there are no published reports on comparing immunoaffinity procedures to recover AFB_1 from spiked ground peanut samples with official AOAC methods. In Table I we detail the results obtained using the two procedures.

TABLE I

COMPARISON OF THE RECOVERY OF AFB_1 FROM SPIKED GROUND PEANUTS USING THE AOAC CB METHOD OR BY IMMUNOAFFINITY CHROMATOGRAPHY USING EASI-EXTRACT COLUMNS

n = Number of samples analysed; S.D. = standard deviation; C.V. = coefficient of variation; EE = EASI-EXTRACT columns.

Method	*Spike concentration (μg/kg)*	*n*	*Recovery (%)*	*S.D.*	*C.V.*
CB	10	39	82.0	17.0	20.7
	50	36	84.1	14.9	17.7
EE	10	34	93.0	12.6	13.6
	50	27	95.5	11.4	12.0

For the CB method we were able to recover 82.0% for the 10-ppb[a] sample and 84.1% for the 50-ppb spiked sample.

For the EASI-EXTRACT immunoaffinity procedure we were able to recover 93.0% AFB_1 from the 10-ppb spiked sample and 95.5% for the 50-ppb sample. These values are significantly higher than the CB method ($p < 0.05$). The coefficients of variation are also lower than those obtained for the CB method indicating the lower variability of the immunoaffinity procedure.

Beside the higher recoveries using the antibody method there is a considerable saving in time of analysis compared with the CB method. Extraction for the former method involves merely blending for 2 min compared with 30 min for the CB method. Much smaller volumes of solvent are required compared with the CB method and there is no intermediary clean up step using silica gel column chromatography. Finally because the antibody recovers AFB_1 specifically there are no interfering spots on the TLC plate enabling easier and more accurate quantification.

We have examined the use of the EASI-EXTRACT columns to recover the other three naturally occurring aflatoxins *viz.* AFB_2, AFG_1 and AFG_2 and have found similar percentage recoveries as those described above for AFB_1 (data not presented). It is our opinion that immunoaffinity clean up columns provide an extremely useful method to improve aflatoxin quantification and accuracy. Whilst the cost of the columns is substantially higher than silica gel columns the savings in time, solvents and the elimination of false-positives because of the specificity of the antibody more than makes up this cost difference. Similar findings to these have been reported for the use of immunoaffinity columns to recover AFM_1 from milk[14] and cheeses[15].

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[a] Throughout the article the American billion (10^9) is meant.

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