

## Determination of Aflatoxins B1 and B2 in Peanuts and Corn Based Products (Penentuan Aflatoksin B1 dan B2 dalam Kacang Tanah dan Produk Jagung)

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### ABSTRACT

*A method for the determination of aflatoxins B1 and B2 in peanuts and corn based products is described. The samples were extracted with a mixture of acetonitrile-water (84:16), followed by multifunctional clean-up and liquid chromatography with fluorescence detection. Both calibration curves showed good correlation from 4.0 to 32.0 ppb for aflatoxin B1 ( $r=0.9999$ ) and 1.2 to 9.6 ppb for aflatoxin B2 ( $r=0.9997$ ). The detection limit of aflatoxins B1 and B2 were established at 0.1 and 0.03 ppb, respectively, based on signal-to-noise ratio of 3:1. Average recoveries for the determination of aflatoxins B1 and B2 at 10 and 3 ppb spiking levels, respectively ranged from 94.2 to 107.6%. A total of 20 peanut samples and corn based products were obtained from retail shop and local market around Kuala Terengganu and analyzed for aflatoxins B1 and B2 contents, using the proposed method. Aflatoxins B1 and B2 were detected in 5 out of the 9 peanuts samples and 5 out of the 11 corn based products, at levels ranging from 0.2 to 101.8 ppb.*

*Keywords: Aflatoxins; corns; multifunctional clean-up column; peanuts*

### ABSTRAK

*Satu kaedah penentuan aflatoksin B1 dan B2 dalam kacang tanah dan produk jagung diuraikan. Sampel diekstrak dengan menggunakan campuran asetonitril-air (84:16), diikuti dengan proses pembersihan dengan turus pelbagai fungsi dan pemisahan melalui kromatografi cecair dengan pengesanan pendarfluor. Kedua-dua lengkung penentuan menunjukkan kolerasi yang baik daripada 4.0 hingga 32.0 ppb untuk aflatoksin B1 ( $r=0.9999$ ) dan 1.2 hingga 9.6 ppb untuk aflatoksin B2 ( $r=0.9997$ ). Had pengesanan ditentukan masing-masing pada 0.1 dan 0.03 ppb untuk aflatoksin B1 dan B2, berdasarkan nisbah isyarat bisingan 3:1. Purata perolehan semula pada aras pemakuan 10 dan 3 ppb masing-masing untuk aflatoksin B1 dan B2 adalah dalam julat 94.2 hingga 107.6%. Jumlah sebanyak 20 sampel kacang tanah dan produk jagung diperolehi dari kedai runcit dan pasaraya tempatan di sekitar Kuala Terengganu untuk dianalisis kehadiran aflatoksin B1 dan B2, dengan menggunakan kaedah yang dicadangkan. Aflatoksin B1 dan B2 telah dikesan dalam 5 daripada 9 sampel kacang tanah dan 5 daripada 11 produk jagung, pada kepekatan dalam julat 0.2 hingga 101.8 ppb.*

*Kata kunci: Aflatoksin; jagung; kacang tanah; pembersihan dengan turus pelbagai fungsi*

### INTRODUCTION

The word mycotoxin was derived from mycotoxicosis, which was a term first used in 1955 to describe diseases of animals caused by toxic metabolic by-products of certain fungi (Herrman 2002) which includes mushrooms, molds and yeast. Mycotoxins can appear in the food chain as a result of fungal infection of crops, either by being eaten directly by human, or used as livestock feed. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain even after heat treatment, such as cooking and freezing (Urraca et al. 2004). The most common mycotoxins are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, T-2 toxin and zearalenone. Of these, aflatoxins represents the main threat world-widely due to their occurrence and toxicity.

Aflatoxins are produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Aycicek et al. 2005). These fungi are present in soil and plant material, cause the decay of stored grain and food. Aflatoxins may increase stress susceptibility and compromise growth efficiency. The clinical signs of aflatoxicosis are extremely varied. Signs of acute aflatoxicosis include depression, nervousness, abdominal pain, diarrhea and death (Herrman 2002).

Aflatoxins occur naturally in most of the commodities, including corn, soybean and peanut which are consumed by human and animal. High moisture and temperature are two main factors that cause the occurrence of mycotoxins at pre-harvest and post harvest stages (Aycicek et al. 2005). Since these toxins have been considered unavoidable contaminants in food chain, the Food and Drug Administration (FDA) of USA has established an action

level for total aflatoxins which is at 20 ppb for all foods, including animal feeds (Munkvold et al. 2005). Malaysia has established the maximum permitted proportions of total aflatoxins in groundnuts and other food which are at 15 and 5 ppb respectively, under Food Regulations 1985 (FoSIM 1985). Of the currently identified 20 aflatoxins, aflatoxin B1 and B2 occur naturally and are significant contaminants of a wide variety of foods and feeds (Juan et al. 2008).

Samples of peanut and corn based product were analysed for the presence of aflatoxins in this study as these food were commonly consumed by Malaysian without any awareness of their safety from any local organizations. The aim of this project was to screen the content of aflatoxins in samples of peanut and corn based products available in Kuala Terengganu that were widely consumed in huge amounts, as well as providing sensitive, accurate and reproducible analytical method for the detection of aflatoxins to assess the exposure of consumers to the toxins in order to bring them to the attention of the importance of monitoring the levels of aflatoxins in the peanuts and corn based products.

## MATERIALS AND METHODS

### CHEMICALS AND REAGENTS

Aflatoxins stock standard consisted of 1000 ppb of B1 and 300 ppb of B2 was purchased from Supelco. Acetonitrile and methanol of HPLC grade were obtained from Merck. Deionized water was obtained from Elga water system.

### PREPARATION OF STANDARD SOLUTIONS OF AFLATOXINS

A serial of working standards ranging from 4 to 32 ppb of aflatoxin B1 (AFB1) and 1.2 to 9.6 ppb of aflatoxin B2 (AFB2) was then prepared by diluting the stock standard solution with methanol. All the standard solutions were stored in dark at 4°C when not in use.

### PREPARATION OF SPIKED PEANUT AND CORN

Uncontaminated peanut and corn based product samples were ground and then split into four 25 g portions for each sample. Three of the portions were spiked with aflatoxins to give final levels of 10 ppb AFB1 and 3 ppb AFB2, respectively whereas the remaining portion was treated as blank.

### CHROMATOGRAPH ANALYSIS

Purified extracts were analyzed by reversed-phase isocratic high performance liquid chromatography (HPLC) from Shimadzu LC 10A using a Platinum C18 column (250 × 4.6 mm id, 5 µm) maintained at 40°C. A fluorescence detector from Shimadzu RF-10AXL was set at 375 nm (excitation) and 440 nm (emission). The mobile phase applied was deionized water/acetonitrile/methanol (60:20:20) with flowrate of 1.0 mL/min and injection volume of 20 µL.

### EXTRACTION AND CLEAN-UP

A 25 g of ground sample was mixed with 100 mL of mixture of acetonitrile and deionized water at ratio of 84:16 and shaken for 1 hour. The extract was filtered through Advantec filter paper No.131. A 9 mL of filtrate was then transferred into a test tube and pressed through Mycosep® #226 AflaZon clean-up cartridge which was obtained from Romer Labs. Inc. (Binder et al. 2007). A 2 mL of purified extract was removed and evaporated to dryness. The dried extract was then reconstituted into 200 µL of methanol, vortexed and filter through 0.45 µm nylon membrane filter prior to HPLC analysis.

### VALIDATION OF ANALYTICAL METHOD

The analytical method was assessed for linearity, recovery, precision and limit of detection before sample analysis.

## RESULTS AND DISCUSSION

The chromatographic conditions were adopted from Supelco Reporter (Yearick & Doughty 1997) with modification. Column temperature of 30, 35, 40 and 45°C and combination of five different excitation and emission wavelengths were studied and optimized in this study in order to increase the sensitivity. The peak area obtained at temperature of 40°C, excitation wavelength of 375 nm and emission wavelength of 440 nm was the highest values for both AFB1 and AFB2 as shown in Figure 1 and 2, respectively. By selecting these optimized parameters, noise and background interference were reduced and hence better sensitivity and lower detection limit were obtained for both toxins.

Five-point graphs were obtained with concentration of 4 - 32 ppb of AFB1 and 1.2 - 9.6 ppb of AFB2. Calibration graphs were drawn by linear regression of the least-squares method using the peak area of standard as response versus concentration as shown in Figure 3. The correlation coefficients were >0.999, which indicated excellent linearity (Christian 2004). The test for the precision of the method was performed by injection repeatability and the results obtained were shown in Table 1. Relative standard deviations for both retention time and peak area of five replicates were less than 5%, which showed high precision for manual injection (Ewing 1997). The detection limits were 0.1 and 0.03 ppb, respectively for AFB1 and AFB2, calculated based on three times the standard deviation of the noise.

The average recoveries for spiked samples ranged from 94.2-107.6% as shown in Table 1. The recoveries obtained were within the range of 70 to 125%, which were acceptable according to Glibert and Anklam (2002) and AOAC International guidelines for method validation (Hall 2008). Figures 4 and 5 showed typical LC chromatograms of clean-up extracts from aflatoxins positive samples in peanut and corn based product. The peaks corresponding to AFB1 and AFB2 were well resolved from background interference peaks with average resolutions of 1.5.

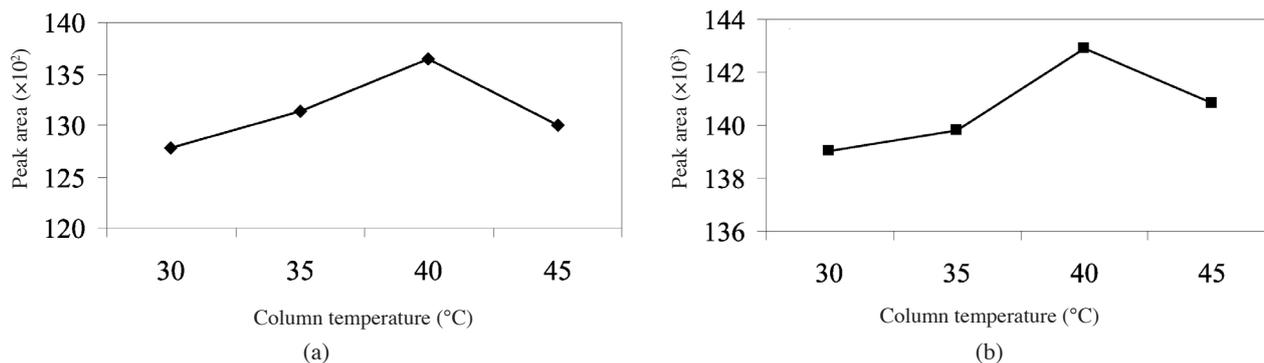


FIGURE 1. Optimization of column temperature to increase chromatographic sensitivity; (a) AFB1 (b) AFB2

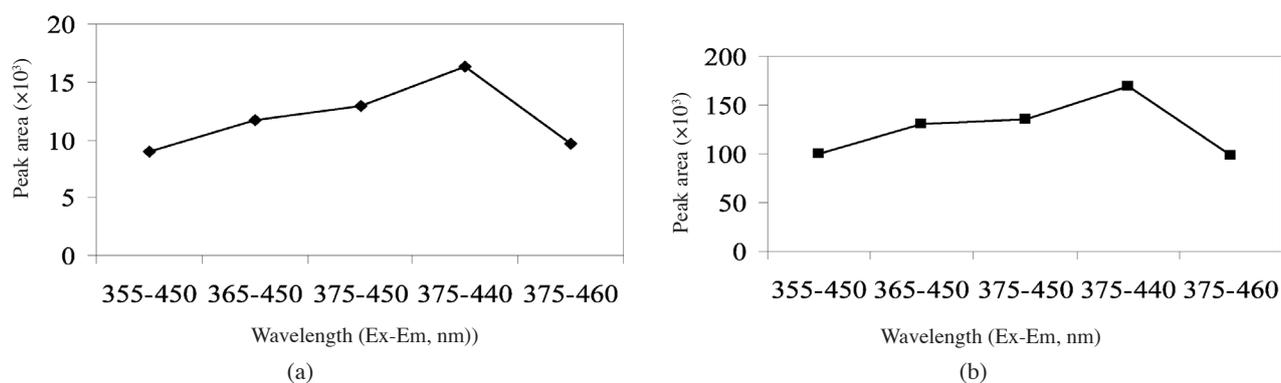


FIGURE 2. Optimization of wavelengths to increase chromatographic sensitivity; (a) AFB1 (b) AFB2

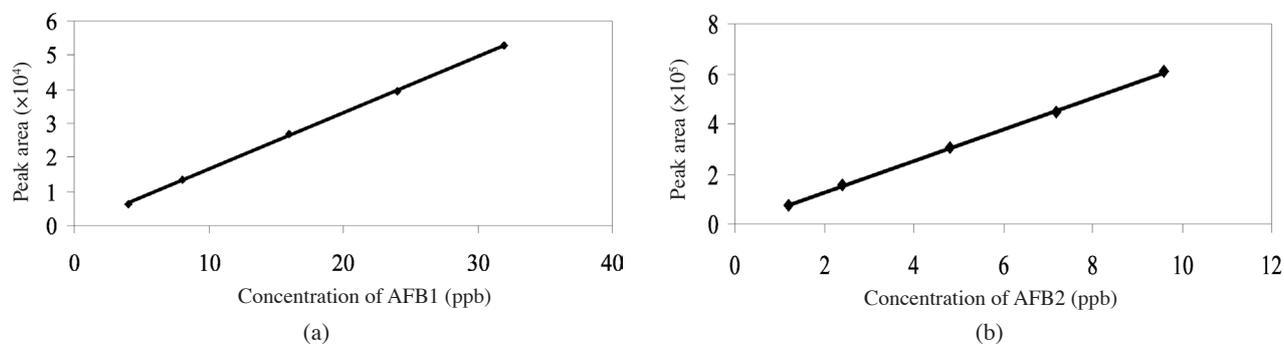


FIGURE 3. Regression curves for (a) AFB1 and (b) AFB2

TABLE 1. Validation data for extraction of AFB1 and AFB2 from peanuts and corns

	Retention time		Peak Area		Recovery from spiked peanuts		Recovery from spiked corns	
	Average (min)	RSD (%)	Average	RSD (%)	Average (%)	RSD (%)	Average (%)	RSD (%)
AFB1	10.9	0.05	12949	1.77	102.2	3.15	94.2	8.73
AFB2	9.3	0.04	154532	3.32	107.6	3.17	98.8	7.39

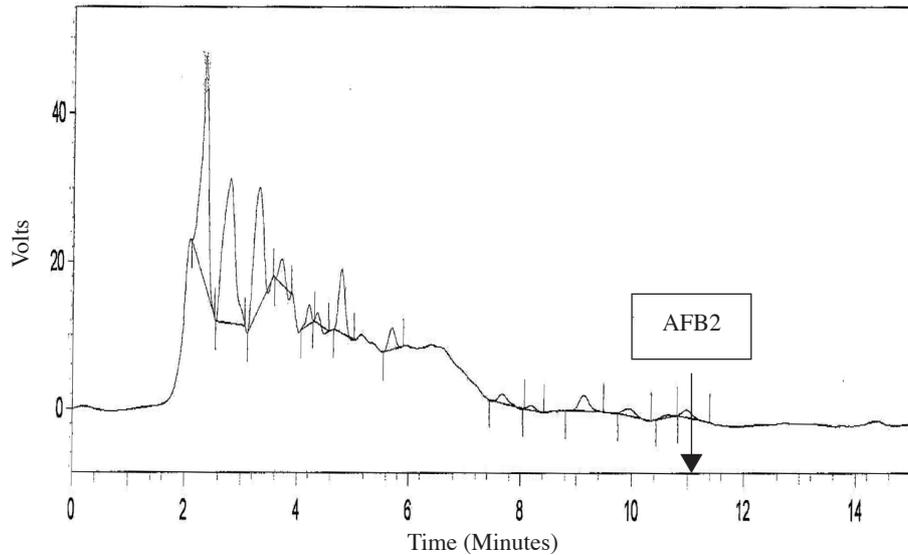


FIGURE 4. Chromatogram of positive peanut sample

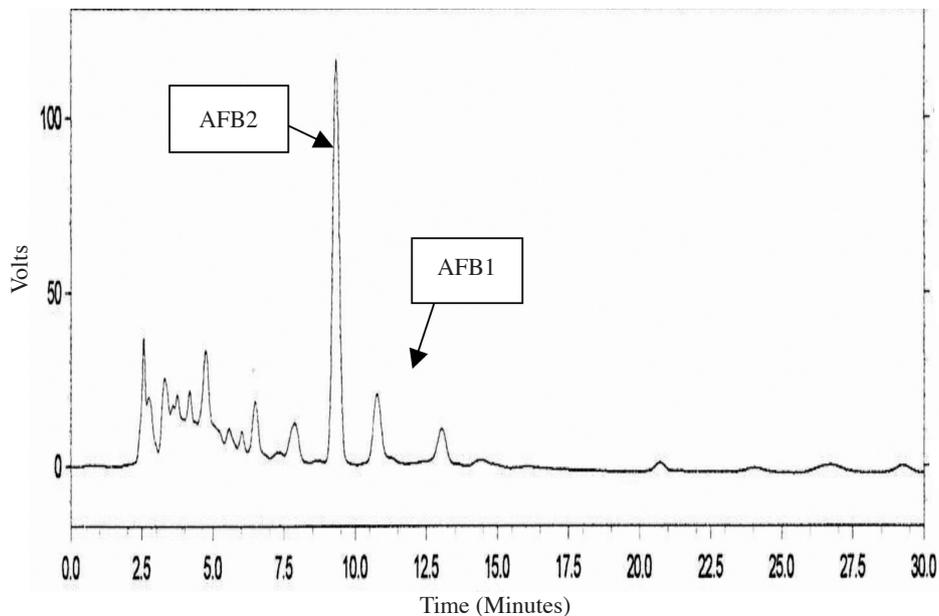


FIGURE 5. Chromatogram of positive corn based product

According to Kazakevich and Lobrutto (2007), baseline separation is achieved for resolution of 1.5 and this is sufficient for complete integration of the desired peak without overlapping with co-eluted peak. From the recoveries and chromatogram obtained, Mycosep® #226 AflaZon clean-up cartridge is proposed to provide sufficient clean-up from impurities and accurate results in determining AFB1 and AFB2 in peanuts and corn based products.

The developed and optimized method was then applied to peanuts and corn based products obtained from selected retail shops and local markets around Kuala Terengganu. The samples selected included those imported, processed and made locally either for human consumption or as

poultry feed. AFB1 was detected in six of the 20 peanut samples and corn based products analyzed in concentrations ranging from 2.5 to 33.4 ppb as shown in Table 2. All the peanut samples selected were for human consumption. All of the positive peanut samples were still within its printed expiry date but exceeded the Malaysia permitted limit, which is 15 ppb for groundnuts. AFB2 was found in seven out of the 20 samples analyzed at concentration of 0.2 to 101.8 ppb. Imported poultry feed labeled as 11, 12 and 13 were all detected with very high concentration of AFB2, where two of them exceeded FDA action levels of 20 ppb. The two popcorn products with sample code of 14 and 15 also exceeded the Malaysia permitted limit in food, which is 5 ppb. Overall, the results revealed

TABLE 2. Concentration of aflatoxins in commercial peanuts and corn based products

Sample type	Sample Code	Concentration (ppb)	
		AFB1	AFB2
Peanut	2	19.5	Not detected
	4	24.8	Not detected
	7	33.4	Not detected
	8	Not detected	0.2
	9	Not detected	0.2
Corn based product	11	3.6	21.0
	12	12.2	101.8
	13	2.5	17.2
	14	Not detected	12.2
	15	Not detected	5.8

that eight out of twenty samples tested in this study had levels of aflatoxins contamination above the Malaysian permitted limits. In other words, the high frequency of aflatoxins contaminations found in both peanuts and corn based products may result in high exposure of aflatoxins for individuals who consume both products regularly.

#### CONCLUSION

Our studies revealed that HPLC coupled with Mycosep® #226 AflaZon clean up cartridge assures good recovery, precision and linearity in the quantitative determination of AFB1 and AFB2 extracted from peanuts and corn based products. This work showed that 40% of both peanuts and corn based products analyzed with the proposed method were positive for aflatoxins that exceeded the maximum tolerable limits set by Malaysia standard. The monitoring and elimination of mycotoxin in consumer products should be implemented and frequently conducted by the industry and government in order to bring the consumers and manufacturers to the concerns of quality and public health.

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