Evaluation of Aflatoxin IAC with Grains and Feed Additives by HPLC and ELISA

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Abstract

Immunoaffinity column (IAC) was developed using anti-aflatoxin monoclonal antibodies for sample clean-up before aflatoxin analysis. Aflatoxins in sample extracts are cleaned up and concentrated by passing through the column before analysis. This IAC/HPLC method was compared with immunoassay (Beacon Aflatoxin Plate Kit; AOAC Performance Tested Method, license No. 081003). Recoveries for IAC/HPLC methods were determined using certified samples (corn and peanut paste). The recoveries ranged from 76% to 84% for corn, and from 81% to 110% for peanut paste. The feed additives such as corn, rice bran, distiller's grains, and feed yeast powder were analyzed by both IAC/HPLC and immunoassay methods. The comparison of IAC/HPLC and immunoassay showed good correlation. Without IAC pretreatment, the immunoassay, slope and r^2 value were 1.31 and 0.9667, respectively. However, when IAC was used to clean up the sample extracts for immunoassay, slope and r^2 value were 0.88 and 0.9995, respectively. This study indicates that the Immunoaffinity column (IAC) pretreatment for the samples reduced the matrix effect and provides good recovery of aflatoxin from grains and feed additives. It can be useful for monitoring aflatoxin contamination in food and feed raw materials by HPLC as well as immunoassay.

Introduction

Aflatoxins are secondary toxic metabolites from the molds, *Aspergillus* species. These molds can grow on corn, peanuts, rice, and other crops. Aflatoxins are carcinogenic to humans, and considered to be one of the most popular mycotoxins. Among more than 16 aflatoxins identified, B1, B2, G1 and G2 are frequently analyzed due to their well known pathogenic action. Also aflatoxin M1 accumulates in the milk of cows that have consumed aflatoxin B1 contaminated feed. Aflatoxin M1 has same toxicity as B1. Purification or clean-up of aflatoxins in samples is always important in terms of increasing sensitivity of analytical method. Liquid-liquid partitioning, solid-phase extraction and immunoaffinity column (IAC) are available. Non-polar cartridges such as (C18, C8, or phenyl) are holding aflatoxins that is eluted by organic solvent. Or the opposite way is to allow aflatoxins passing through while the interfering substances are retained (MycoSep). The most modern clean-up tool is the immunoaffinity column (IAC). Since it has a specific binding ability to aflatoxins, no other interferences will be collected. Therefore, IAC has become a major tool for aflatoxin analysis. In this study, a monoclonal antibody specific to alfatoxins B1, B2, G1, and G2 was developed and evaluated by ELISA (AOAC PTM) and HPLC (AOAC 991.31).

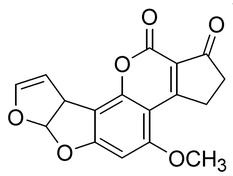

Figure 1. Structure of aflatoxin B1.

Methodology

Monoclonal antibody production:

Immunized to BALB/c mice with AFB-BSA conjugate. Booster injections were performed. Spleen cells were fused to make hybridoma. Colonies were screened by checking the reactivity with aflatoxin B1, B2, G1 and G2.

Immobilization in gel:

Purified monoclonal antibodies were immobilized to CNBr-activated Sepharose 4B gel. Immobilization procedure was conducted based on the instruction of the Sepharose gel.

Extraction and purification by IAC:

Sample (25 g) + NaCl (5 g) + MeOH/H2O (70/30, 125 mL) -> Blending for 2 min

Filter through paper filter. Take 15 mL and mix with 30 mL of H_2O. Filter through glass fiber filter.

Take 15 mL of the filter extract and load on the affinity column.

Let it pass by gravity. Pass 20 mL H_2O for washing. Blow out the remaining H_2O.

Elute out the aflatoxins with 1 mL of MeOH. Use it for analysis.

ELISA analysis:

Beacon Aflatoxin Plate Kit; AOAC Performance Tested Method, license No. 081003

HPLC analysis:

AOAC method (991.31). Agilent 1200 series with fluorescence detector (Excitation: 365 nm, Emission: 435 nm), PHRED reactor (25 m X 0.25 mm id size coil, Aura Industries, Inc.), Column: Columbus C18 (150 X 4.6 mm, 5u, Phenomenex), Mobile phase: water-acetonitrile-methanol (50-15-35). Flow rate: 0.7 mL/min.

Results

Table 1 Recovery of afaltoxins analyzed by HPLC with IAC

Corn				Peanut paste			
Level (ng/g)	Determined (ng/g)	Yield (%)	Total yield (%)	Level (ng/g)	Determined (ng/g)	Yield (%)	Total yield (%)
B1 (6.53)	5.04	77	76	B1 (15.7)	16.07	102	110
B2 (2.09)	1.65	79		B2 (0.6)	0.85	141	
G1 (2.28)	1.79	79		G1 (2.9)	4.14	143	
G2 (2.2)	1.53	69		G2 (0.3)	0.42	1401	

Table 2 Total aflatoxins determined by HPLC and ELISA

	Corn	Rice bran	Distiller's grain	Hydrolyzed protein powder
HPLC with IAC	145	20	28	55
ELISA with IAC	162	21	27	58
ELISA w/o IAC	130	29	54	62

Table 3 Cross reactivity of anti-aflatoxin monoclonal antibody

Aflatoxin	Cross reactivity (%)
B1	100
B2	10
G1	31
G2	28

Discussion

A monoclonal antibody against aflatoxins was evaluated by HPLC (AOAC official method) and ELISA (AOAC PTM). Its cross reactivity to B1 is 100%. But, cross reactivities to B2, G1 and G2 are 10, 31 and 28%, respectively. Although it has low cross reactivity toward B2, G1 and G2, those three aflatoxins are present in relatively small amount. For the IAC procedure, 20 mL of water was used to wash out interference compounds. This doesn't cause the loss of aflatoxins. Two reference materials of corn and peanut paste were tested to determine the recoveries. Recoveries of individual aflatoxin (B1, B2, G1 and G2) from corn sample ranged from 69 to 79% resulting in a total yield of 76%. The recoveries from peanut paste ranged from 102 and 143% resulting in a total yield of 110%. Even though, these two recoveries do not match 100%, they are within the acceptable ranges. Also, the results from real samples analyzed by HPLC and ELISA using IAC or no IAC were compared. Using IAC, the results of HPLC and ELISA were pretty close each other. However, the result of ELISA without using IAC had higher values from rice bran bai, distiller's grain, and ferment phthalocyanine powder. Only the corn sample gave lower values without using IAC. This might be explained by matrix effects either suppressing or enhancing from non-IAC ELISA testing because the extract solution to be tested is still turbid even after dilution. This reflects the IAC purification gives a merit to avoid matrix effect that may cause either signal suppression or enhancement.

An evaluation of immunoaffinity column for aflatoxins was successfully performed. This IAC could be used for any aflatoxin analysis format such as HPLC and ELISA.

References

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