

## ► CLEAN-UP AND DETERMINATION OF AFLATOXINS IN PEANUT AND PEANUT BUTTER

Wendy Rasmussen, Maria Ofitserova, PhD

### BACKGROUND

Aflatoxins occur naturally in peanuts, cottonseed, corn, and dried chili pepper as well as many mixed or processed foods and feeds. Of significant assistance is the cleanup of extracts by an Immunoaffinity column containing antibodies specific to the Mycotoxin of interest. We used a simple, sensitive and robust HPLC method with post-column photochemical derivatization and fluorescence detection was used to analyze Aflatoxins B1, B2, G1, G2 in peanut butter and ground peanuts. The UVETM (LCTech, Germany) photochemical reactor requires no additional reagents and is easy to install between the HPLC column and FLD detector. This method and instrumentation allows for quick and interference-free detection of Aflatoxins at the low ppb level.



### PROJECT OVERVIEW

As part of a NIST study we analyzed samples of peanuts and peanut butter (table 1, 2). Four other laboratories that use other HPLC methods for analysis of Aflatoxins participated in this study. Community results for peanuts are presented in table 2. The extracts were cleaned up using the AflaCLEAN™ (LCTech, Germany) Immunoaffinity columns for Aflatoxin B1, B2, G1, G2.

For the handling of the columns, we used the AcceCLEAN automated system that processes three samples simultaneously. The prepared samples were analyzed by HPLC with post-column Photochemical derivatization using the UVE Photochemical Reactor.



### METHOD

#### Isolation of Aflatoxins B1, B2, G1, G2

Blend 20 g of sample at high speed with extraction solution (100 mL of Methanol/water 80/20, 50 mL of Hexane, 2 g NaCl) and filter through fluted paper. Dilute 14 mL of aqueous layer with 86 mL of PBS buffer (pH7.2), filter and apply 11 mL of solution on AflaCLEAN™ Immunoaffinity column. The toxins are eluted with 2 mL of Methanol and analyzed as described.

#### Analytical Conditions

Analytical Column: Mycotox™

(Pickering Laboratories, Inc), C<sub>18</sub>, 4.6x250 mm

HPLC Eluent: Sodium Phosphate buffer  
(Cat #1700-1108)/Methanol/  
Acetonitrile (57/28/15)

Flow Rate: 1 mL/min

Injection Volume: 30 µL

FLD: Excitation 365 nm, Emission 430 nm



Placement of UVE™ (Note: HPLC Model shown is to demonstrate placement of the UVE™ only. No endorsement expressed or implied.)

## RESULTS &amp; DISCUSSION

The 6-point calibration curves were built in a range of 11.49 – 0.24 ppn for B1, 3.29 – 0.07 ppb for B2 and G2, 9.85 – 0.21 ppb for G1 with  $R^2$  exceeding 0.999.

There were no matrix interferences were present after the sample clean up using the Immunoaffinity columns, and using the AcceCLEAN, we were able to cleanup 30 samples automatically in just a few hours.

The results for all Aflatoxins are in good agreement with certified NIST values and with the results obtained by other methods.

Using the IAC columns, AcceCLEAN to handle the columns, and the UVE reactor to derivatize, we were able to detect low levels of Aflatoxin quickly and efficiently.

**Table 1. Peanut butter (NIST SRM2387) – control sample**

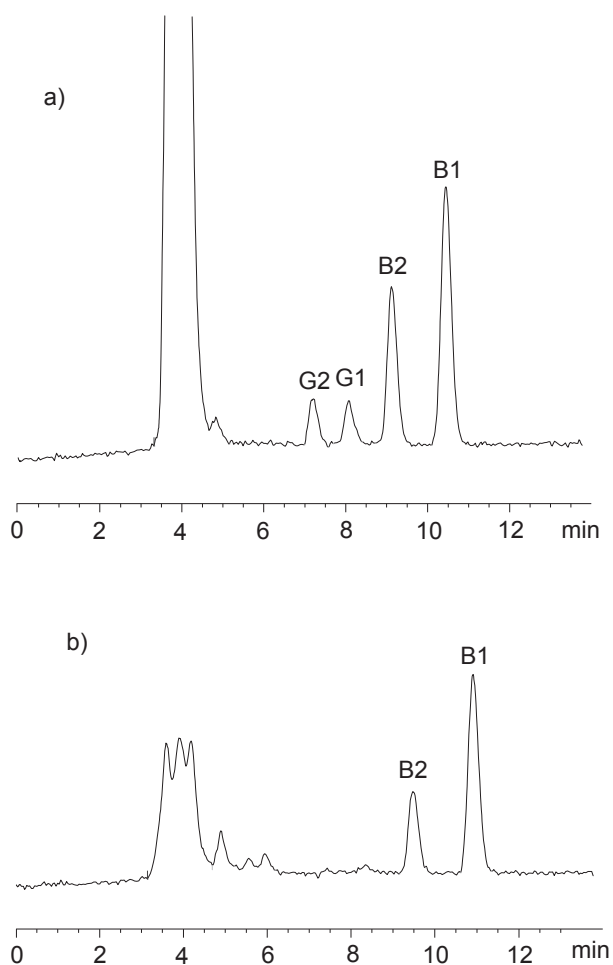
	Aflatoxin B1	Aflatoxin B2	Total Aflatoxins
Target value, ng/g	4.2 ± 0.9	0.7 ± 0.3	5.0 ± 0.5
Packet A, ng/g	4.47	0.73	5.2
Packet B, ng/g	4.76	0.96	5.72
Packet C, ng/g	4.74	0.8	5.54

**Table 2. Ground peanut sample**

	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Total Aflatoxins
Packet A, ng/g	6.21	1.82	1.74	1.24	11.01
Packet B, ng/g	6.45	1.65	2.02	1.3	11.42
Packet C, ng/g	5.73	1.78	2.07	1.52	11.1
Mean, ng/g	6.1	1.8	1.9	1.4	11.2
RSDr %	6.5	5.6	10.5	7.1	1.8
Community results*	4.02-6.48	1.38-1.75	1.54-2.22	1.34-1.45	8.4-11.6
NIST assessed value**, ng/g	7.47 ± 3.28	1.82 ± 0.79	2.57 ± 1.13	1.64 ± 0.72	13.5 ± 5.9

\* Results from 5 participating laboratories are presented as (minimum reported value – maximum reported value), ng/g

\*\* ± 95 % confidence interval about the NIST assessed value



**Fig. 1 Chromatograms of a) Ground peanuts; b) NIST SRM2387 peanut butter sample. All samples are part of NIST Exercise E (April 2010).**