PICKERING LABORATORIES

Clean-Up and Determination of Aflatoxins in Peanuts and Peanut Butter

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 to analyze Aflatoxins B1, B2, G1, G2 in peanut butter and ground peanuts. The UVE™ (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 participants in an 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. The prepared samples were analyzed by HPLC with post-column photochemical derivatization using the UVE™ Photochemical Reactor.

Method

Isolation of Aflatoxins B1, G1, B2, G2

To 20 g of sample add 2 g of NaCl, 100 mL of extraction solution (80/20 Methanol/water) and 50 mL of Hexane. Blend at high speed and filter through fluted paper. To 14 mL of aqueous layer add 86 mL of PBS buffer pH 7, mix well and filter. Open the Afla-Clean Immunoaffinity column and drain the storage buffer. Load 11 mL of extract/PBS mixture and drain to the top of the sorbent bed. Wash the column with 10 mL of water. Elute Aflatoxins with 2 portions of 1 mL Methanol. Leave the first portion of Methanol in the column for 5 min before adding the second portion to ensure the breaking of the Aflatoxins bond with the antibodies. Analyze Aflatoxins as described below.

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

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

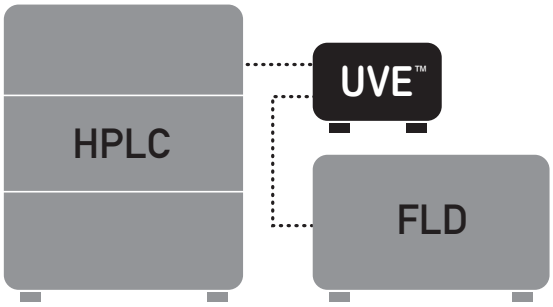
Results & Discussion

Isolation of Aflatoxins B1, G1, B2, G2

The 6-point calibration curves were built in a range of 11.49 – 0.24 ppb for B1, 3.29 – 0.07 ppb for B2 and G2, 9.85 – 0.21 ppb for G1 with R2 exceeding 0.999. There were no matrix interferences present after the sampleclean-up using the Immunoaffinity columns.

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, and the UVE reactor to derivatize, we were able to detect low levels of Aflatoxin quickly and efficiently.



Flow diagram for UVE™ Photochemical Reactor

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*

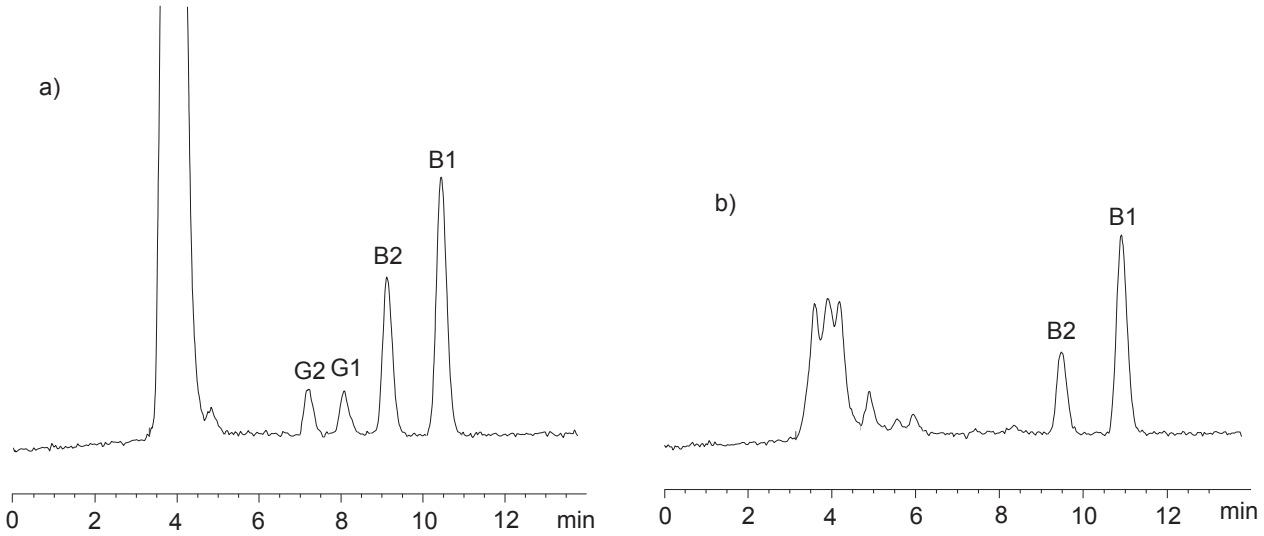


Fig 1. *Chromatograms of a) Ground peanuts; b) NIST SRM2387 peanut butter sample. All samples are part of NIST Exercise E (April 2010).*