APPLICATION BRIEF

Food/Nutraceutical

Determination of Aflatoxins in Raw Peanuts by UHPLC/SQ MS

Aflatoxins are harmful or fatal to livestock and are considered carcinogenic to animals and humans. They are secondary metabolites produced by *Aspergillus Flavus* and *Aspergillus Parasiticus* fungi. Aflatoxins can accumulate in agricultural products such as cereals, peanuts, dried fruits, wine, etc. due to inadequate storage conditions. Several aflatoxins have been identified and the most ubiquitous ones are B1, B2, G1 and G2. We describe a UHPLC/SQ MS method to identify these aflatoxins in ground peanuts using immunoaffinity cartridges for sample clean up.

Experimental Conditions

Target Analytes: Aflatoxins B1, B2, G1, G2

Sample Preparation Conditions

A fine powder of raw peanuts (5 g) was spiked with aflatoxin G2 (20 ng/g) and aflatoxins B1, B2 and G1 (5 ng/g). A methanol/water (60:40) mixture (25 mL) was added to the sample and mixed thoroughly for 10 min. A control sample of ground peanuts containing no spike of aflatoxins was prepared similarly. The samples were centrifuged at 2,000 RPM for 10 min. The supernatant was passed through a 0.45 µm filter and an aliquot of the filtered solution (5 mL corresponding to 1 g of peanuts) was mixed with an equal volume of water.

The sample was loaded on an immunoaffinity cartridge (Alfaprep, R-Biopharm, Darmstadt, Germany) at a flow of 1 mL/min. The immunoaffinity cartridges contained a gel suspension of monoclonal antibodies specific for aflatoxins B1, B2, G1 and G2. The cartridge was then washed with water (5 mL). Aflatoxins were eluted from the cartridge with methanol (1 mL). The cartridge was backflushed during elution to improve recovery. The sample was brought to about 0.5 mL and reconstituted to 1 mL with water and injected on the analytical system.

Liquid Chromatography Conditions

Pump Type: PerkinElmer® Flexar™ FX-15

Column: PerkinElmer Brownlee™ HRes C18 column (2.1 mm x 50 mm, 1.9 µm)

Mobile Phase: A: water with 0.1% formic acid
B: 50/50 acetonitrile/methanol containing 0.1% formic acid

Flow Rate: 0.5 mL/min

Injection Volume: 3 µL in partial fill mode

Gradient:

Time (min)	%A	%B
0	75	25
2.5	60	40
1	60	40

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Mass Spectrometer Conditions

Ionization: Ultraspray™ ESI – Positive mode

The $[M+H]^+$ ions for each of the aflatoxins were monitored in three different time periods:

Time Period 1: (0-2.1 min) SIM ion 331.0 for G2; dwell time of 300 ms

Time Period 2: (2.1-2.7 min) SIM ions 329.0 and 315.0 for G1 and B2 respectively; dwell time of 200 ms each

Time Period 3: (2.7-3.5 min) SIM ion 313.0 for B1; dwell time of 300 ms

Capillary Exit Voltage: 100 V

Results

This application's detection limits for each of the aflatoxins B1, B2 and G1, G2 by UHPLC/SQ MS was estimated at 0.4 ng/mL (S/N >3). Calibration curves of all the aflatoxins showed excellent linearity for a concentration range of 0.5 ng/mL. A representative calibration curve for aflatoxin B1 is shown in Figure 1. The recovery of aflatoxin G2 (20 ppb) spiked in peanut samples as internal standard and purified over the immunoaffinity cartridge was estimated at 72 ±10.7% (n = 3). The recovery of spiked aflatoxins B1, B2 and G1 in the peanuts using G2 as internal standard was estimated in the range of 90-128%. Figure 2 shows the SIM ions of the aflatoxins analyzed in the peanut samples with and without spike of each of these analytes.

The FDA guidelines recommend an action level of 20 ppb of total aflatoxins in peanuts and other cereals for both human and animal consumption. With the Flexar SQ 300 MS it was possible to easily quantify 5 ppb of aflatoxins in peanuts using immunoaffinity clean up, fully addressing FDA requirements reaching sensitivity similar to fluorescence detection.

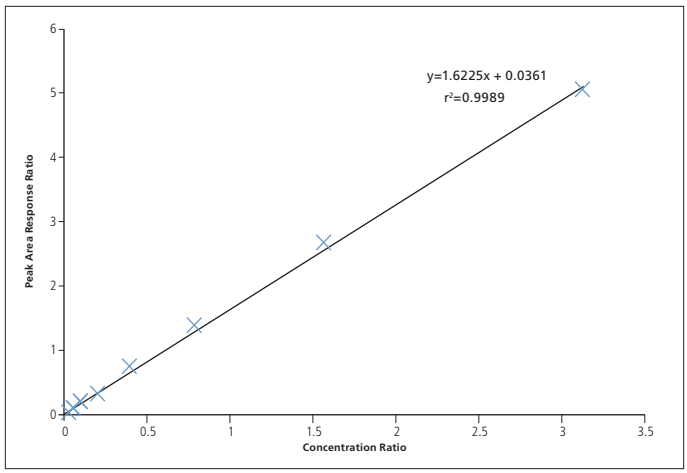

Figure 1. Calibration curve for aflatoxin B1 (0.48-63 ng/mL) using aflatoxin G2 as internal standard (20 ng/mL).

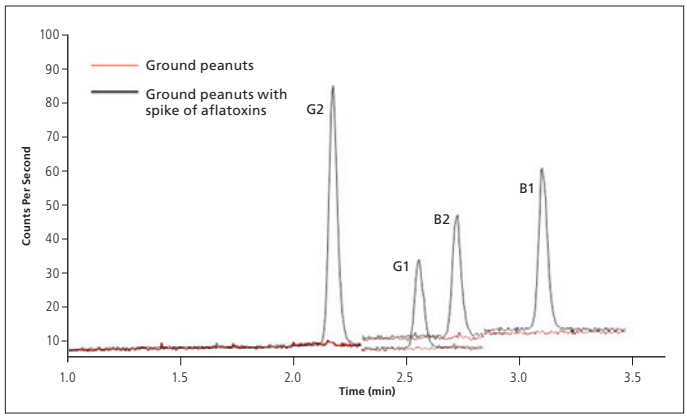

Figure 2. Overlaid SIM chromatograms of $[M+H]^+$ ions of aflatoxins B1, B2, G1 in ground peanuts spiked at 5 ppb level with aflatoxin G2 used as internal standard (spiked at a concentration of 20 ppb). A control sample of ground peanuts with no spike of aflatoxins is also shown

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