

Matrix of the Month

Oktober 2014:

Aflatoxin B/G in Pumpkin Seeds



Do you have a special matrix that we should test for mycotoxins?
Please let us know and write an e-mail to info@LCTech.de!

Protocol

Spike 20 g homogenized pumpkin seed with 2 g sodium chloride and extract them with 100 mL methanol/water (80/20 (v/v)) and 50 mL n-hexane for 5 to 10 minutes.

Filtrate the extract and centrifuge it for 10 minutes at 2000 xg facilitating the phase separation between the diluted/aqueous and the n-hexane phase. Dilute 10,5 mL of the aqueous phase (the lower phase) with 64,5 mL PBS buffer (pH 7.2) and filtrate the diluted extract with a fibre glass filter to delete any turbidities.

Apply 50 mL to the immunoaffinity column AflaCLEAN or Afla-OtaCLEAN and rinse afterwards the sample reservoir with 10 mL of de-ionised water. Apply this washing solution onto the IAC column too.

Dry the column and elute with methanol. Pay attention that the methanol incubates within the column bed for 5 minutes to ensure that the antibody is entirely denaturated and the toxin is released.

Dilute and measure the eluate to HPLC conditions.

HPLC Conditions

Aflatoxin B, G

HPLC: Isocratic

Column oven: 36 °C

Separation column: RP C18 (e.g. P/N 10522)

Flow rate: 1.2 mL/min, water/methanol/acetonitrile (60/30/15 (v/v/v))

Fluorescence detection with post column derivatisation (photochemical with UVE)

Excitation wavelength: 365 nm

Emission wavelength: 460 nm

Recovery Rates

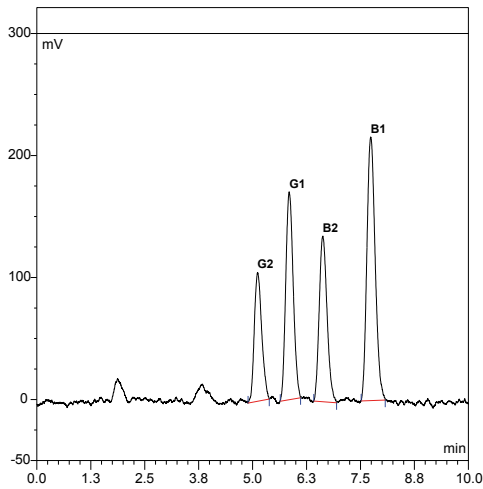
Content of Aflatoxins B1, B2, G1 and G2 in Pumpkin Seeds

Aflatoxin	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery rate** Pumpkin seeds 10 ppb (10 ppb = 10µg/kg)	102	106	107	102

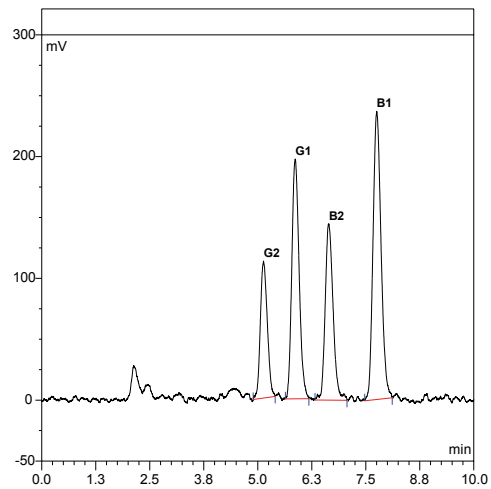
* Standard is set = 100 % , ** corrected with non-spiked sample



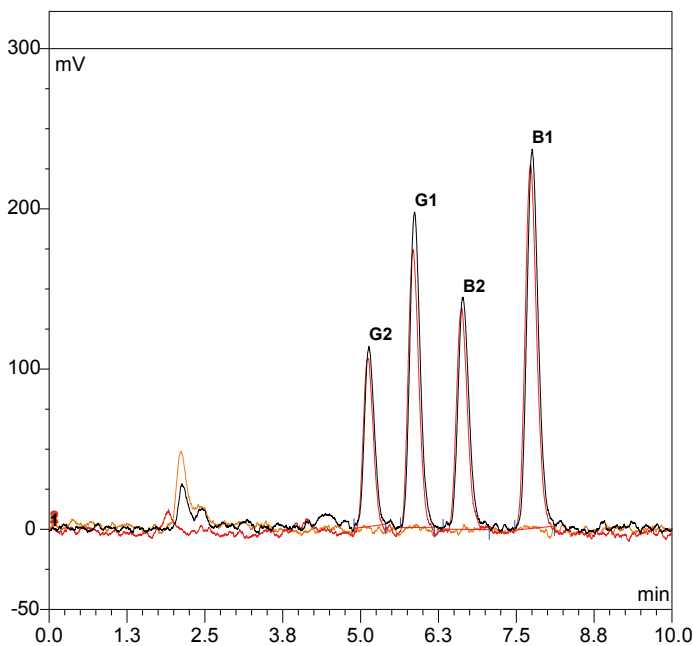
Chromatograms



Aflatoxin, standard 14 ng / 2 mL



Pumpkin seed, spiked with 10 ppb Aflatoxin



Overlay of chromatograms
Aflatoxin, standard 14 ng / 2 mL (red),
Pumpkin seed, spiked with 10 ppb Aflatoxin (black),
Pumpkin seed, not spiked (orange)

These LCTech products were used:

AflaCLEAN,
Immunoaffinity column
for Aflatoxin B1, B2, G1, G2

P/N 10514 / 11721

Afla-OtaCLEAN,
Combined column
for Aflatoxin and Ochratoxin

P/N 11022 / 11771

UVE,
Photochemical reactor
for the analysis of Aflatoxins

P/N 10519

HPLC column,
for mycotoxin analysis

P/N 10522

Do you have further questions?
Please just write an e-mail to info@LCTech.de