



July 2020

Aflatoxin B/G and Ochratoxin A in Peanut ~ Manual and Automated for LC-MS/MS with CrossTOX® ~

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

Sample Preparation

MYCOTOXINS

Peanut

Botanically speaking, the peanut is a legume which is related to the bean or pea. Because of its net-like and woody shell, the peanut is understood and traded as a nut. The consistency, the high oil content and the comparatively low starch content as well as the raw edibility also distinguish the peanut from legumes such as beans. Today's main producers are China with 16 billion tons per year followed by India, Nigeria, USA and Sudan.

Further processing of peanuts or incorrect storage conditions may result in the formation of mycotoxins, which are toxic to humans and animals even in low amounts. For this reason, the European Union applies strict import controls. In 2020 alone, 79 out of a total of 184 complaints by the RASFF portal in Europe in the food and feed sector have already been registered for peanut products (as of 19.06.2020).

Automated processing with the CrossTOX® Multi-Mycotoxin Clean-up Column

The CrossTOX® columns by LCTech allow a high efficient sample clean-up of all regulated plus expected mycotoxins. At the same time, the columns improve the conventional dilute-and-shoot process via an QuEChERS based procedure. The columns are suitable for both manual and automated processing.

For automated processing of the CrossTOX® columns the FREESTYLE system offers two options:

- from raw extract to cleaned sample or
- from raw extract to chromatogramm (full automation)

In order to realize the highest possible degree of automation, the FREESTYLE SPE robotic system is simply upgraded with an HPLC Direct-Injection module for direct injection into your LC-MS/MS system. This enables the parallelization of clean-up and analysis for up to 120 samples/day and thus to an optimum utilization of your LC-MS/MS system.



Processing Protocol

Homogenise 20 g of peanut and extract the sample with 100 mL acetonitrile/water/acetic acid (84/15/1 (v/v)) and 50 mL n-hexane to remove fats and oils. For a high extraction efficiency, continue the extraction for at least 10 minutes.

Filter the raw extract. A phase separation between n-hexane and acetonitrile-containing phase can be accelerated by centrifugation at 3000 x g for 5 minutes. Let pass 0.5 - 3 mL of the lower (n-hexane-free phase) slowly through the CrossTOX[®] column, collecting the cleaned sample using a GC-vial. A volume between 250 - 500 µL is sufficient to obtain a sample that contains a maximum depletion of matrix components.

Recovery Rates

Content of Aflatoxin B1, B2, G1 and G2 in Peanut

Aflatoxin	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery rate** Peanut 10 ppb (B1/G1/B2/G2 (4/4/1/1)) (n=3)	99 ± 5	97 ± 7	88 ± 2	97 ± 9
Recovery rate** Peanut 20 ppb (B1/G1/B2/G2 (8/8/2/2)) (n=3)	93 ± 5	94 ± 7	92 ± 7	88 ± 6
Recovery rate** Peanut 40 ppb (B1/G1/B2/G2 (16/16/4/4)) (n=3)	97 ± 3	102 ± 9	91 ± 5	96 ± 5
Recovery rate** Peanut 50 ppb (B1/G1/B2/G2 (20/20/5/5)) (n=3)	98 ± 3	96 ± 8	92 ± 2	103 ± 8

* Standard was set = 100%, ** Corrected with unspiked sample / The results comply with the performance specifications of EC 401 / 2006 (Section 4.3.1).

Recovery Rates

Content of Aflatoxin B1, B2, G1 and G2 in Peanut

	Ochratoxin A
Standard*	100
Recovery rate** Peanut 10 ppb (n=3)	89 ± 8
Recovery rate** Peanut 20 ppb (n=3)	95 ± 7
Recovery rate** Peanut 40 ppb (n=3)	91 ± 8
Recovery rate** Peanut 50 ppb (n=3)	88 ± 3

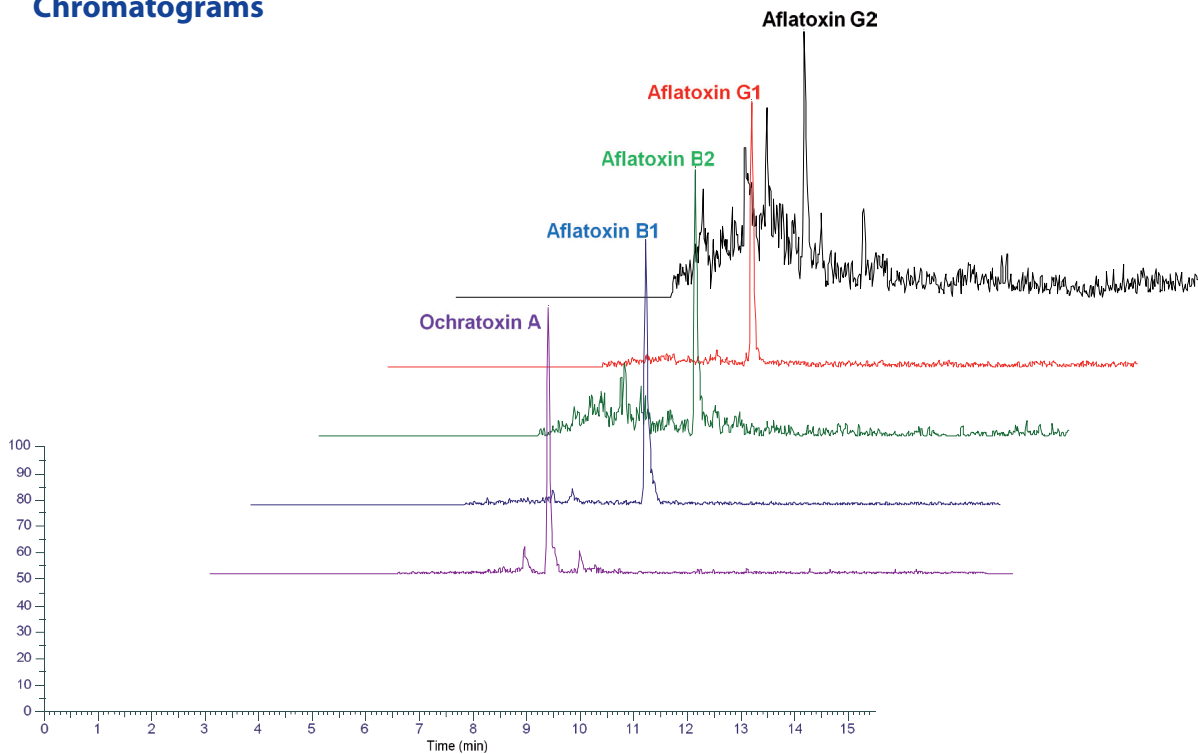
* Standard was set = 100%, ** Corrected with unspiked sample / The results comply with the performance specifications of EC 401 / 2006 (Section 4.3.1).

LC-Conditions

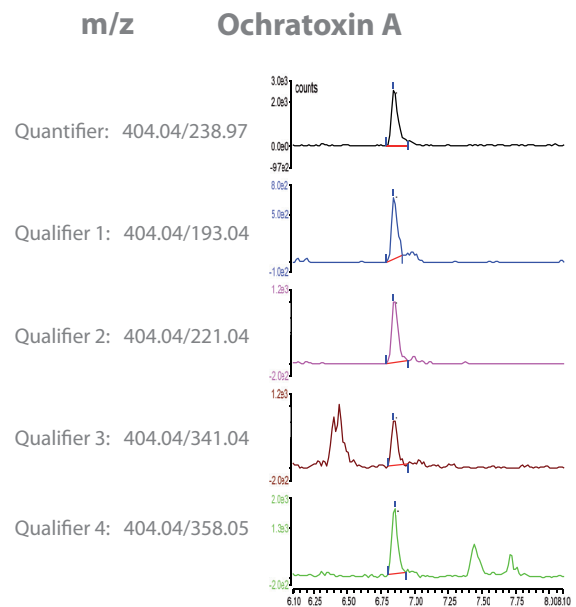
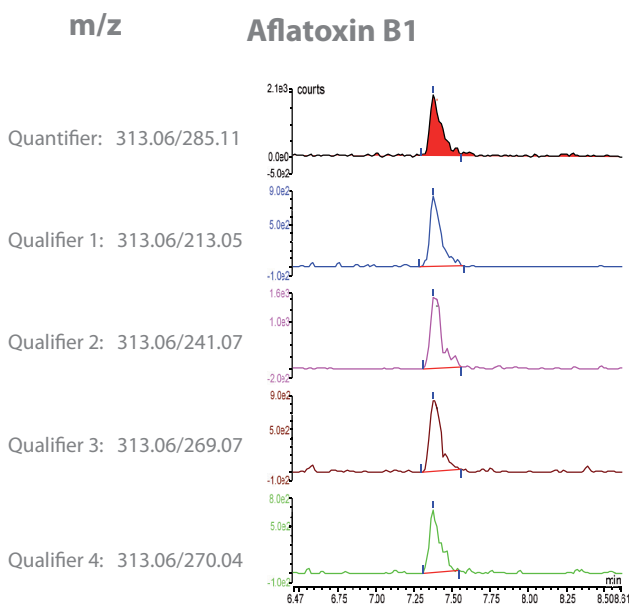
Aflatoxine B/G and Ochratoxin A

LC:	Gradient
Column Oven:	38 °C
Separation Column:	Accucore Biphenyl 100 mm x 2.1mm; 2.6 µm with Defender Guard
Flow Rate/Eluent:	Eluent A: HPLC water/methanol (98/2 (v/v))+ 1 % acetic acid + 5mM ammonium acetate Eluent B: HPLC water/methanol (2/98 (v/v))+ 1 % acetic acid + 5mM ammonium acetate Flow rate 0.4 mL/min
Mass Spectrometry QQQ:	For all analytes at least 1 quantifier and 3 qualifier ions were used, the settings depend on the MS device.

Chromatograms



Chromatography for Aflatoxin B1 and Ochratoxin A of the Selected Qualifiers and Quantifiers



These LCTech Products were used:

Multi-Mycotoxin Column CrossTOX®
P/N 17901 / 17902

FREESTYLE SPE Robotic System for
Automated Sample Preparation
P/N 12663 / 12668