



January 2019

## Aflatoxin B/G and Ochratoxin A in Turkey Rearing Feed ~ Manual and Automated ~

*Do you have a special matrix that we should test for mycotoxins? Please let us know and write an e-mail to: [mycotoxins@LCTech.de](mailto:mycotoxins@LCTech.de)*

### Sample Preparation

MYCOTOXINS

#### Turkey Meat

Goulash, skewer or steak - turkey meat is one of the most popular meat in Germany. It is not only versatile and easy to prepare, but also a supplier of valued protein, essential iron, B vitamins and minerals. Further, it contains a high proportion of niacin and thus stimulates the metabolism. Furthermore, turkey meat is low in fat and calories.

To ensure the good quality of our meat and thus animal health, the feed, as in this case turkey rearing feed, plays a decisive role. Mycotoxins in animal feed can lead to significant losses in yield and quality of agriculture, as they have a major influence on animal health. Feeding composition could contain mycotoxins by cereal dust or low quality ingredients.

#### Automated Sample Preparation with FREESTYLE SPE

Since 1998, LCTech has been developing and supplying products and methods for the preparation and analysis of food, animal feed and environmental samples. Thereby we can continue to consume our food with a clear conscience.

The product range includes semi- and fully automated sample preparation systems as well as consumables for the analysis of contaminants and residues. The automated robotic system FREESTYLE SPE offers unique possibilities for sample preparation by solid phase extraction (SPE).

Any manual SPE method that has already proven itself in the laboratory can be automated quickly and easily. Simply carry out the preparatory processing steps as described on the following page. Then position the sample in the FREESTYLE SPE, parameterise the method in the software with a few mouse clicks and start the system - done.



## Processing Protocol

Homogenise 10 g of matrix (turkey rearing feed) and add 1 g of sodium chloride. Extract the sample through 50 mL of methanol/water (80/20 (v/v)) and add 25 mL n-hexane in order to remove fat and essential oils. For high extraction efficiencies, continue the extraction for 10 minutes.

Filtrate the raw extract and dilute 10 mL of it with 64.5 mL of PBS. In case of precipitations, filtrate the sample again.

Load 50 mL of the sample (corresponding to 1.4 g matrix) onto an Afla-OtaCLEAN column for clean-up of both toxins at once or individually depending on the toxin onto an AflaCLEAN or OtaCLEAN column. Wash the column with 10 mL of deionised water each and dry it afterwards by flushing air through it.

Elute the toxin with 2 mL of methanol. The column bed is incubated with methanol for 5 minutes to ensure a fully denaturation of the antibodies and release of toxins.

Dilute the sample to eluent conditions and measure it afterwards via HPLC with fluorescence detection or LC-MS.

## HPLC-Conditions (Aflatoxin B/G / Ochratoxin A)

Mykotoxin:	Aflatoxin B/G	Ochratoxin A
HPLC:	isocratic	isocratic
Column Oven:	36 °C	40 °C
Separation Column:	RP C-18 (P/N 10522)	RP EC 125/3 120-3 C18 or RP C-18 (P/N 10522)
Flussrate:	1.2 mL/min	0.6 mL/min
Flow Rate:	HPLC-Water/Methanol/Acetonitrile (60/30/15 (v/v/v))	HPLC-Water/Methanol/Acetonitrile (40/55/5 (v/v/v)) + 1 % Acetic Acid
Flourescence Detection:	Derivatisation with UVE Photochemical Reactor	Without Derivatisation
Excitation Wavelength:	365 nm	335 nm
Emission Wavelength:	460 nm	465 nm

## Recovery Rates

Content of Aflatoxin B/G in Turkey Rearing Feed

Aflatoxin	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Turkey Rearing Feed, 20 ppb (Afla-OtaCLEAN)	100	98	104	91
Recovery Rate** Turkey Rearing Feed, 20 ppb (AflaCLEAN)	102	100	102	90
Recovery Rate** Turkey Rearing Feed 40 ppb (Afla-OtaCLEAN)	94	93	98	92
Recovery Rate** Turkey Rearing Feed 40 ppb (AflaCLEAN)	94	94	100	87

\*Standard is set = 100 %, \*\*Corrected with non-spiked sample /  
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)

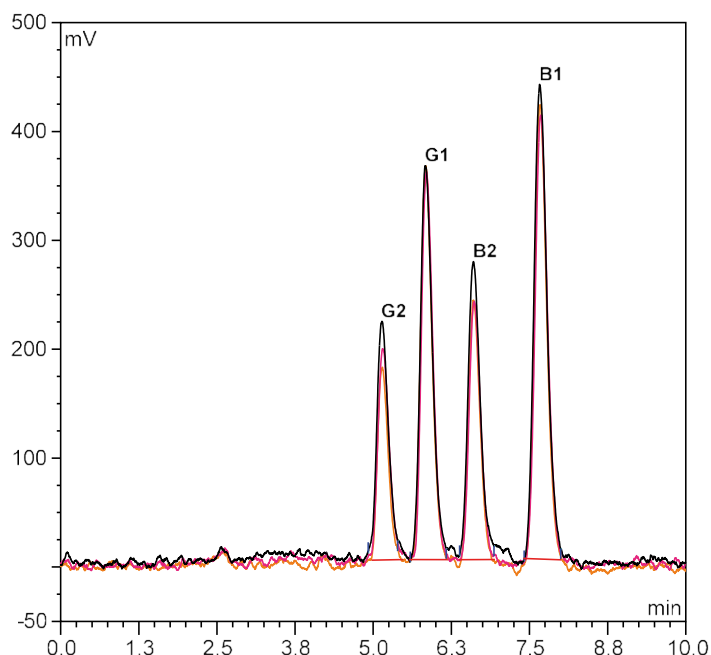
## Recovery Rates

Content of Ochratoxin A in Turkey Rearing Feed

Mykotoxin	Ochratoxin A
Standard*	100
Recovery Rate** Turkey Rearing Feed, 20 ppb (Afla-OtaCLEAN)	91
Recovery Rate** Turkey Rearing Feed, 20 ppb (OtaCLEAN)	87
Recovery Rate** Turkey Rearing Feed 40 ppb (Afla-OtaCLEAN)	91
Recovery Rate** Turkey Rearing Feed, 40 ppb (OtaCLEAN)	88

\*Standard is set = 100 %, \*\*Corrected with non-spiked sample /  
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)

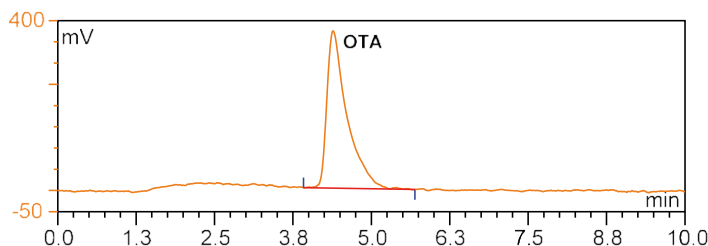
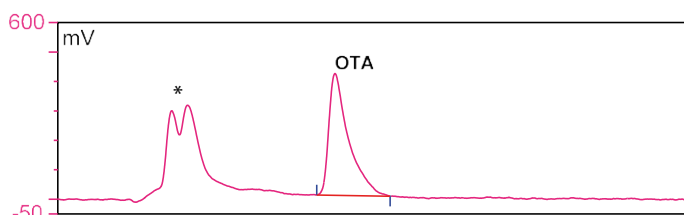
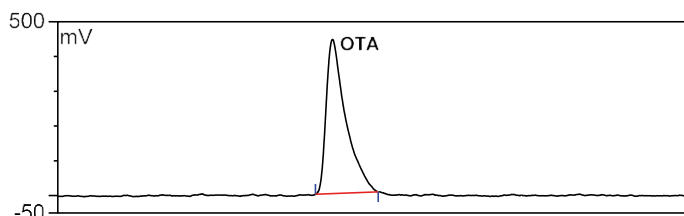
## Chromatograms



**Black:** Standard 40 ppb = 16 ppb B1/G1 and 4 ppb B2/G2 (56 ng / 2 mL)  
**Red:** 40 ppb tukrey rearing feed cleaned-up with Afla-OtaCLEAN  
**Orange:** 40 ppb tukrey rearing feed cleaned-up with AflaCLEAN



AflaCLEAN, OtaCLEAN and Afla-OtaCLEAN column



**Black:** Standard 40 ppb (56 ng / 2 mL OTA)  
**Red:** 40 ppb tukrey rearing feed cleaned-up with Afla-OtaCLEAN  
**Orange:** 40 ppb tukrey rearing feed cleaned-up with OtaCLEAN

\* Additional signals by the simultaneously copurified aflatoxins B/G

## Time and Money Cleverly Saved!

The chromatograms show that good recoveries and excellent chromatography results can be achieved with the LCTech immunoaffinity columns even in highly contaminated batches.

For the clean-up of aflatoxins B/G and ochratoxin A in one matrix, Afla-OtaCLEAN also halves the working time and saves money at the same time, as both toxin groups can be cleaned-up in one step with the combined immunoaffinity column.

### These LCTech Products were used:

Afla-OtaCLEAN Immunoaffinity Columns for  
 Ochratoxin A and Aflatoxin B/G  
 P/N 11022 / 11771

AflaCLEAN Immunoaffinity Columns for Aflatoxin B/G  
 P/N 10514 / 11721

OtaCLEAN Immunoaffinity Columns for Ochratoxin A  
 P/N 10515 / 11535

HPLC Separation Column RP C-18  
 P/N 10522

UVE Photochemical Reactor  
 P/N 10519