



September 2019

## Aflatoxin B/G and Ochratoxin A in Cumin ~ 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

#### Cumin

Cumin – a wonder herb?! Cumin is mainly found in China, India and South America. It is more taste-intensive as the caraway, which is mostly used for cooking in European countries. Both species grow on parallel branches of the umbellifer.

The high content of essential oils also helps to improve digestion and trigger a growth-inhibiting effect on bacteria and fungi. To digest indigestible foods, it used to be common to eat caraway seeds with a little sugar after a heavy, fatty meal.

In order to preserve cumin, it is imported into Europe in dried form from the growing regions. During this drying process or under incorrect storage conditions, mycotoxins can be formed, which can reduce the quality of the spice and cause damage to human health. For this reason, there are strict EU-wide controls on imports from third countries.

#### The Immunoaffinity Column Afla-OtaCLEAN for Clean-up of Mycotoxins

Clean-up of difficult matrices, such as coffee or spices, presents many challenges in everyday laboratory work and cost a lot of time. To facilitate these steps, LCTech developed the immunoaffinity column Alfa-OtaCLEAN. With the combined column, you can clean-up Aflatoxin B/G and Ochratoxin A simultaneously in all matrices, as well as cumin, quickly and with good recovery rates.

The mycotoxins Aflatoxin B/G and Ochratoxin A are often found combined in the food and feed sector. With the Afla-OtaCLEAN column, you can clean-up both mycotoxins in only one step. This saves you half the money and half of the time.



Immunoaffinity Column  
Afla-OtaCLEAN

... and how quick and easy you can transfer manual methods to automation, you will find out at [www.LCTech.de](http://www.LCTech.de)

## Processing Protocol

Homogenise 10 g of cumin and add 2 g of sodium chloride. Extract the mixture with 100 mL methanol/water (80/20 (v/v)) and add 50 mL n-hexane in order to remove fat and essential oils.

To ensure high extraction efficiencies, continue the extraction for at least 20 minutes. Centrifugation may help to separate n-hexane phase as top layer from the methanolic bottom layer. Filtrate the raw extract and dilute 2 mL with 12 mL PBS (contains 8 % Tween20). In the next step load sample (corresponds to 0.2 g matrix) onto the immunoaffinity column Alfa-OtaCLEAN.

Subsequently, dry the column with a short airflow. Elute the column with 2 mL of methanol. Keep in mind that the column bed is incubated with methanol for 5 minutes in order to ensure a fully denaturation of the antibodies and release of toxin.

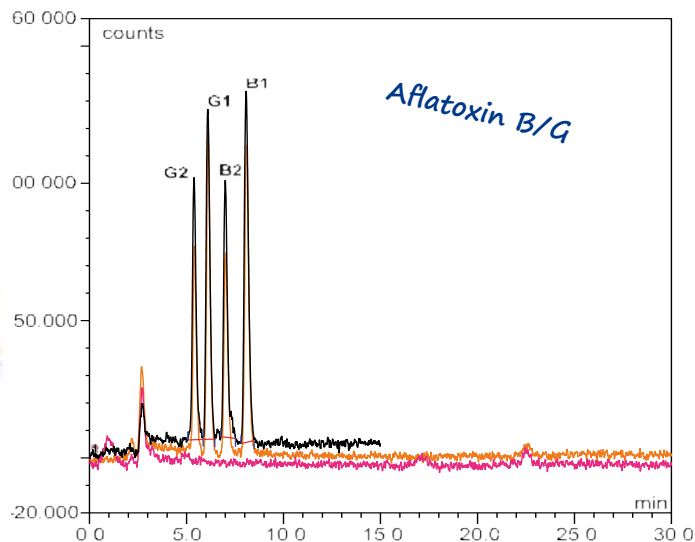
HPLC-Conditions (Aflatoxin B/G)	
Mycotoxin	<b>Aflatoxin B/G</b>
HPLC:	isocratic
Column Oven:	36 °C
Separation Column:	RP C-18 (P/N 10522)
Flow Rate:	1.2 mL/min
Eluent:	HPLC-Water/Methanol/ Acetonitrile (60/30/15 (v/v/v))
Flourescence Detection:	Derivatisation with UVE Photochemical Reactor
Excitation Wavelength:	365 nm
Emission Wavelength:	460 nm

HPLC-Conditions (Ochratoxin A)	
Mycotoxin	<b>Ochratoxin A</b>
HPLC:	isocratic
Column Oven:	40 °C
Separation Column:	RP EC125/3 nucleosil 120-3 C18
Flow Rate:	0.6 mL/min
Eluent:	HPLC-Water/Methanol/ Acetonitrile (40/55/5 (v/v/v)) + 1 % acetic acid
Flourescence Detection:	Without Derivatisation
Excitation Wavelength:	335 nm
Emission Wavelength:	465 nm

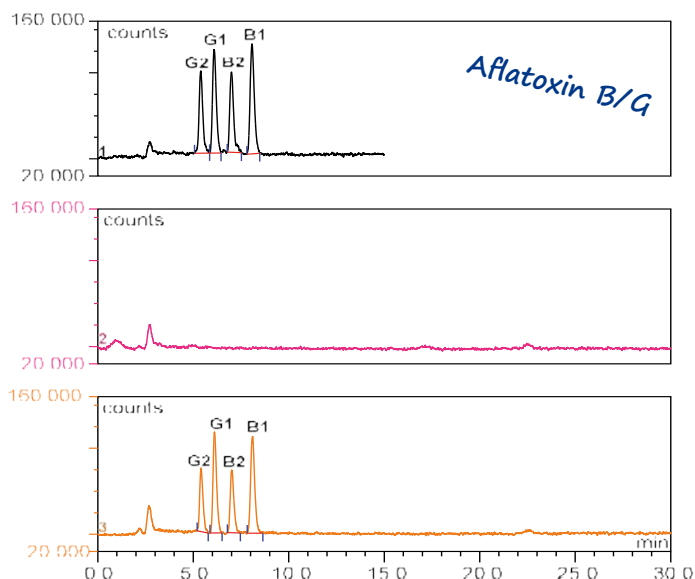
Recovery Rates Content of Aflatoxin B/G and Ochratoxin A in Cumin						
Mycotoxins	B1	B2	G1	G2	OTA	Σ
Standard*	100	100	100	100	100	<b>100</b>
Recovery Rate** Cumin, 20 ppb (Afla-OtaCLEAN)	83	79	88	72	83	<b>81</b>

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

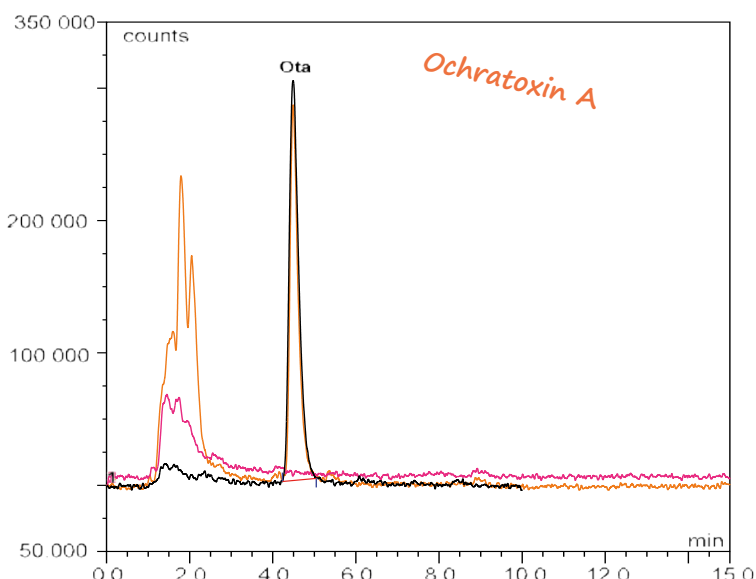
## Chromatograms



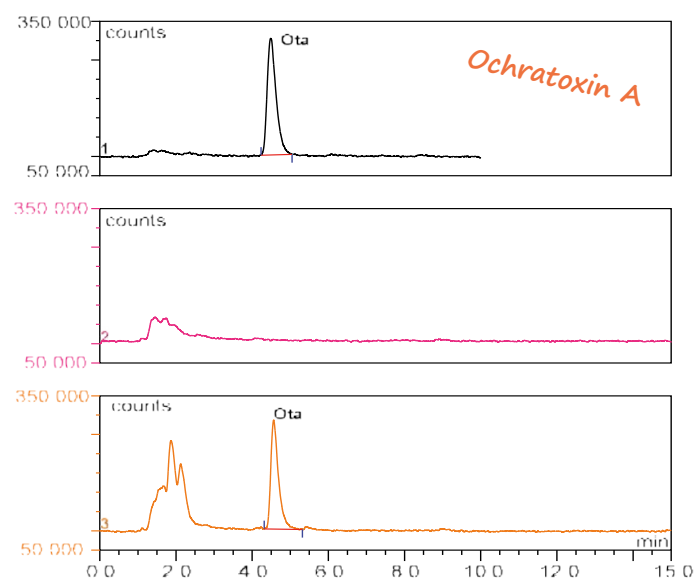
Black: Standard 4 ng / 2 mL  $\hat{=}$  20 ppb total toxin  
 Red: Cumin not spiked  
 Orange: Cumin spiked with 20 ppb



**Black:** Standard 4 ng / 2 mL  
**Red:** Cumin not spiked  
**Orange:** Cumin spiked with 20 ppb



**Black:** Standard 4 ng / 2 mL  $\hat{=}$  20 ppb toxin  
**Red:** Cumin not spiked  
**Orange:** Cumin spiked with 20 ppb



**Black:** Standard 4 ng / 2 mL  
**Red:** Cumin not spiked  
**Orange:** Cumin spiked with 20 ppb

## „2 in 1“ with the Afla-OtaCLEAN column

- **Good recovery rates** even in highly contaminated areas
- You **save money and half of the time** as you only need one column for clean-up of Aflatoxin B/G and Ochratoxin A.
- Suitable for **automated processing** e.g. with FREESTYLE SPE



### These LC Tech Products were used:

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

HPLC Separation Column RP C-18  
 P/N 10522

UVE Photochemical Reactor  
 P/N 10519