



January 2020

Aflatoxin B/G and Ochratoxin A in Tea ~ 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: info@LCTech.de

Sample Preparation

MYCOTOXINS

Tea

Tea is one of the best known and most popular hot drinks along with coffee. The most famous are the real teas, „black tea“ and „green tea“, both made from leaves of the tea plant (*Camellia sinensis*). There is also a wide range of tea-like products such as fruit and herbal teas in different compositions. With more than 2 million tons of tea harvested in 2017, which corresponds to around 40 % of the total, China is by far the world's largest tea producer.

The harvested blossoms and leaves of the tea plant are dried before further processing and then exported for the most part. Mycotoxins can form during this drying process or under incorrect storage conditions. These are toxic to humans and lead to health damage or even death. For this reason there are EU-wide regulations and controls which set the limit value for mycotoxins and thus ensure high product quality.

Two in one - Combined immunoaffinity column Afla-OtaCLEAN

Aflatoxins B/G and Ochratoxin A are naturally occurring mycotoxins and are formed by fungi when products are stored moist or incorrectly. They are often found together in many food and feed products. In order to facilitate extraction and halve the working time, it is advisable to examine the extracts for several mycotoxins in a single step. For this reason LCTech offers the combined immunoaffinity column Afla-OtaCLEAN for the simultaneous clean-up of aflatoxin B1, B2, G1, G2 and ochratoxin A.

Since LCTech produces both, the clean-up columns and the antibodies, extensive quality tests throughout the entire production process ensure high product quality.

On the following page you will find a processing protocol with the use of an Afla-OtaCLEAN immunoaffinity column.



Immunoaffinity Column Afla-OtaCLEAN,
for Aflatoxins B/G and Ochratoxin A

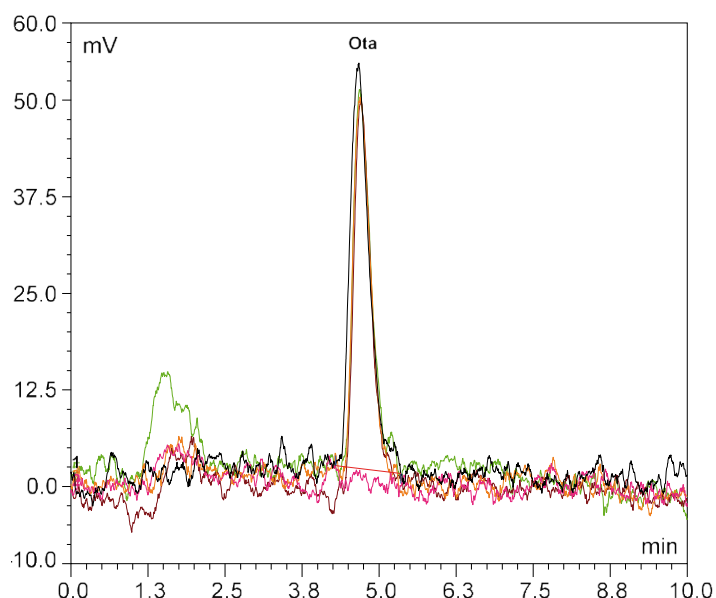
Processing Protocol

Homogenise 10 g of tea and add 2 g sodium chloride to the sample. Then extract the mixture through 100 mL methanol/water (80/20 (v/v)) and 50 mL n-hexane, to remove essential oils and fat. To ensure high extraction efficiencies, perform the extraction for at least 30 minutes. Use the n-hexane free phase for the further processing.

Filter the raw extract and dilute 2 mL with 12 mL PBS (contains 8 % Tween). Afterwards load the sample (14 mL, corresponding to 0.2 g matrix) onto an Afla-OtaCLEAN immunoaffinity column.

Wash the column with 10 mL of deionized water, the washing solution is used to rinse out the template vessel before applied onto the columns to remove residue matrix from the column. Elute the column with 2 mL methanol. Make sure that the column bed is incubated with methanol for 5 minutes in order to ensure a fully denaturation of the antibodies and release of the toxin.

The complete clean-up process can be performed fast and easy by using the robotic system FREESTYLE SPE. Position the tea sample in the FREESTYLE SPE. Change settings or parameters in the software with a few mouse clicks and start the system.



Black = Standard 2ng/2mL OTA (correspond 10 ppb)
Red, orange, brown and green: Individual
AflaOtaCLEAN column eluate of black tea

The chromatogram shows that with the LCTech immunoaffinity columns Afla-OtaCLEAN good recoveries, excellent chromatography results and high reproducibility can be achieved even in highly contaminated samples.

HPLC-Conditions (Alfatoxin B/G / Ochratoxin A)

Mycotoxin	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 nucleosil 120-3 C18
Flow Rate:	1,2 mL/min	0,6 mL/min
Eluent:	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 Ochratoxin A in Tea

Mycotoxin	Ochratoxin A
Standard*	100
Recovery Rate** Black tea, 10 ppb	94
Recovery Rate** Green tea, 10 ppb	90
Recovery Rate** Camomile tea, 10 ppb	87
Recovery Rate** Oriental spiced tea, 10 ppb	94
Recovery Rate** „Winter time“ tea, 10 ppb	95

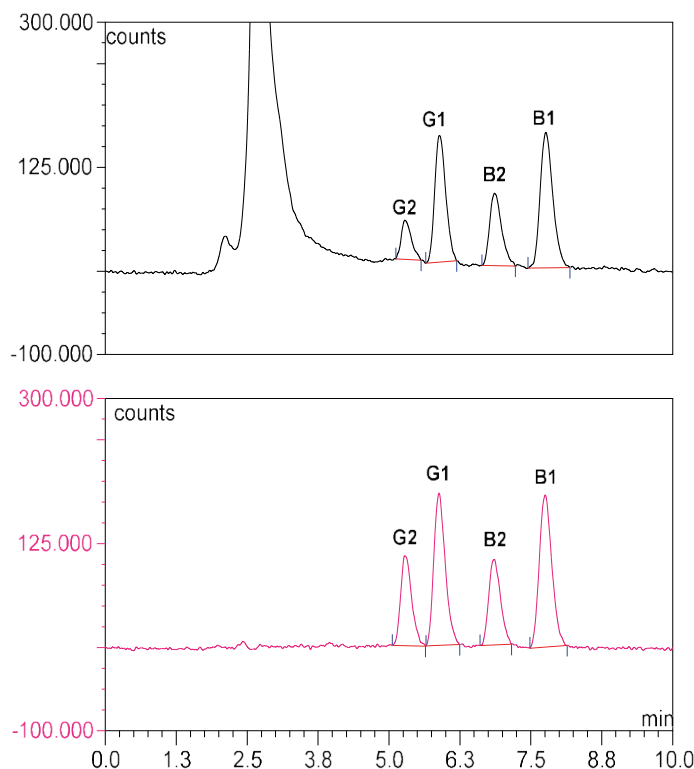
*Standard is set = 100 %, **Corrected with non-spiked sample/
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)

Recovery Rates

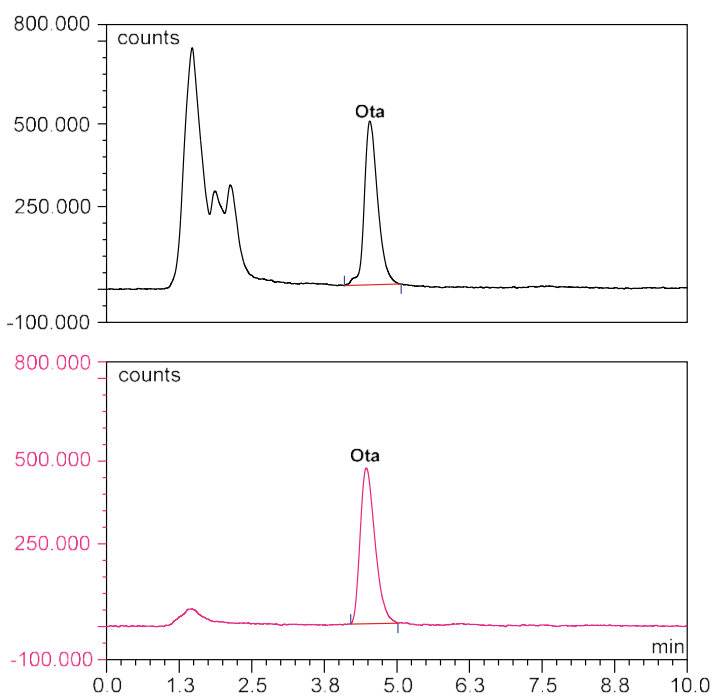
Content of Aflatoxins B1, B2, G1, G2 in Tea

Aflatoxin B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Black tea, 10 ppb	91	88	84	75
Recovery Rate** Green tea, 10 ppb	89	87	85	73
Recovery Rate** Camomile tea, 10 ppb	95	94	88	83
Recovery Rate** Oriental spiced tea, 10 ppb	89	93	87	86
Recovery Rate** „Winter time“ tea, 10 ppb	96	93	97	67

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



Black = Green tea, cleaned-up with LCTech immunaffinity column AflaOtaCLEAN
Red = Standard 10 ppb total (4 ppb (B1/G1)), (1 ppb (B2/G2)) Aflatoxin B/G (2ng/2mL)



Schwarz = Green tea, cleaned-up with LCTech immunaffinity column AflaOtaCLEAN
Rot = Standard 10 ppb (2 ng/2 mL)

Conclusion

The recovery rates and chromatographic data show efficient toxin enrichment despite a difficult matrix, such as tea, which can affect clean-up efficiency through fermentation processes as well as colorants and flavors. No strong matrix interferences are visible within the 10 minutes shown here. Therefore the chromatography time can be reduced to less than 10 minutes. The use of Afla-OtaCLEAN columns allows a fast, reproducible analysis of tea and other matrices. Interfering matrix effects are so effectively depleted that sensitive analysis is possible far below the legally regulated level.

These LCTech 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