



October 2019

Aflatoxin B/G and Ochratoxin A in Pistachios ~ 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

Sample Preparation

MYCOTOXINS

Pistachios

Whether salty, as ice cream, as pistachio paste or as noble decoration on pralines - we encounter pistachios in the most varied forms. The stone fruit belongs to the sumac family. Already in classical antiquity, it was appreciated as a delicacy, in the Roman Empire it was even used as a remedy. The origin of pistachios is not known, but Iran, the USA and Turkey are among the largest growing areas. The fruit consists for the most part of unsaturated fatty acids and has a large proportion of B vitamins. Pistachios are particularly susceptible to the development of mycotoxins as they provide a good basis for the growth of moulds whose metabolites can have toxic effects.

Drying or incorrect storage conditions promote the indication of mycotoxins. Therefore, a too high proportion of mycotoxins may be toxic to humans. For this reason, the European Union carries out strict import controls. If the mycotoxin content is too high, they are returned to the country of origin.

Combined Analysis of Ochratoxin A and Aflatoxin B/G

Aflatoxin B/G and ochratoxin A are often found together in many food and feed products. To facilitate the extraction of the two mycotoxins, LCTech developed the Afla-OtaCLEAN columns for combined clean-up. The Afla-OtaCLEAN columns are available in the useful 3 mL format. Additional, LCTech offers miniaturized SMART columns.

You can put the immunoaffinity columns AflaCLEAN SMART and OtaCLEAN SMART on top of each other and clean-up aflatoxins B/G and ochratoxins A in one step.

On the following page, you will find a processing protocol for the use of 3 mL Afla-OtaCLEAN columns as well as AflaCLEAN SMART or OtaCLEAN SMART columns.



SMART column and 3 mL column

Processing Protocol

Homogenise 20 g of pistachios with 100 mL methanol/ water (80/20 (v/v)). To ensure high extraction efficiencies, continue the extraction by using an Ultra-Turrax 3 - 5 minutes or by using a magnetic stirrer for 10 - 20 minutes. Filter the raw extract and dilute 7 mL of the n-hexane free phase with 43 mL PBS. Filtrate the diluted extract through a Whatman glass fiber filter to remove turbidities.

AflaCLEAN SMART and OtaCLEAN SMART column:

Stick the AlfaCLEAN SMART column over the Male/Female-Luer connector onto the OtaCLEAN SMART column. The columns can be combined pressure-tight by the special connection. Load 10 mL sample (corresponds to 0.28 g matrix) with a maximum flow rate of 1.5 mL/min onto the column. Flush the sample container with 2 mL of deionized water and load the rinsing solution also onto the column.

Elute the AflaCLEAN SMART and OtaCLEAN SMART column with 400 µL of methanol. The elution of both columns at the same time is possible, as long as the analysis provides for simultaneous measurement of the two toxin groups. Keep in mind that the eluent is applied to the column bed for at least 5 minutes before collecting the eluate. Dry the column with a short airflow and collect any remaining eluate.

Alfa-OtaCLEAN column:

Load 50 mL of the sample onto the 3 mL Afla-OtaCLEAN column (corresponds to 1.4 g matrix). Rinse the sample container with 10 mL of deionized water and load the rinsing solution onto the column. Dry the column bed with a short airflow and elute the toxins with 2 mL methanol. Make sure that the methanol incubates in the column bed for 5 minutes to ensure a fully denaturation of the antibodies and release of the toxin.

Conclusion:

Compared to the 3 mL Afla-OtaCLEAN column, the AflaCLEAN SMART and OtaCLEAN SMART column give you a smaller volume. However, the concentration remains the same. This not only saves solvents, but also money and time.

HPLC-Conditions (Aflatoxin 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 Aflatoxin B/G in Pistachios

Aflatoxine B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Pistachios, 10 ppb Afla-OtaCLEAN	86	108	110	102
rel. error (%)	3	9	5	4
Recovery Rate** Pistachios, 10 ppb AflaCLEAN SMART	93	108	97	97
rel. error (%)	3	5	3	4

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

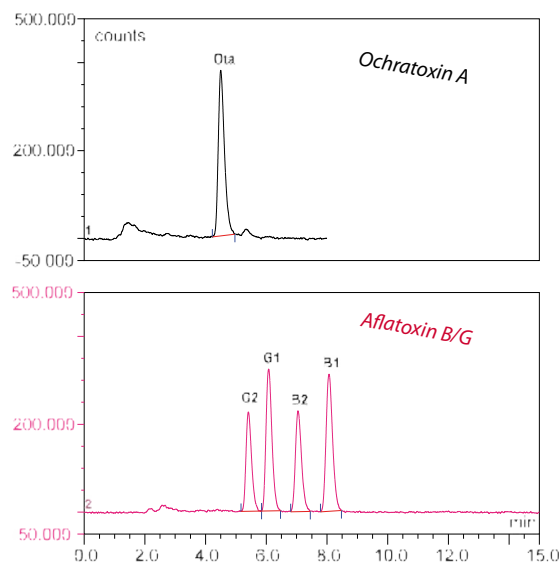
Recovery Rates

Content of Ochratoxin A in Pistachios

Mycotoxin	Ochratoxin
Standard*	100
Recovery Rate** Pistachios, 10 ppb Afla-OtaCLEAN	86
Recovery Rate** Pistachios, 10 ppb OtaCLEAN SMART	90
rel. error (%)	4

*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



Pistachios, cleaned-up with 10 ppb



Small + Fast + Economical = SMART

- Suitable for manual and automated processing, e.g. with the FREESTYLE ThermELUTE™ robotic system
- Significant reduction of processing time to less than 20 minutes for essential clean-up steps from raw extract to eluate
- Saving of up to 80 % of solvents
- Very good recoveries for all matrices
- Reproducible results
- Better chromatography and data analysis
- Handy format of SMART columns

These LCTech Products were used:

OtaCLEAN SMART Immunoaffinity Column for Ochratoxin A
P/N 13346 / 13351

AlfaCLEAN SMART Immunoaffinity Column for Aflatoxin B/G
P/N 12862 / 12863

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

UVE Photochemical Reactor
P/N 10519