Matrix of the Month

April 2020

A□atoxin B/G and Ochratoxin A in Hemp
~ Manual and automated ~

Do you have a special request, which matrix you would like us to test for you? Please contact us by e-mail to: info@LCTech.de

Sample Preparation

MYCOTOXINS

Hemp

Hemp, also known as cannabis, is one of the oldest useful and ornamental plants in the world. As early as 10,000 BC, hemp was used as a medicinal plant in China and is therefore often referred to as „Panaceas", which means „all healer". That is why hemp is also often used for pain relief in diseases such as AIDS, cancer, MS and rheumatism. For this reason the acquisition and the intake by prescription is legalized in some countries like Canada, USA, Uruguay, etc. The individual components of the plant are universally applicable and can be used to produce many different products, such as tea.

Hemp flower tea has a variety of health-promoting and calming properties. For example, it reduces the symptoms of migraines or helps with sleep disorders. In addition, tea has a purifying effect and curbs the appetite, which is the reason why it is often used to accompany diets.

By modifying the flowers, the THC contained in the plant material is converted into CBN and thus no longer has an intoxicating effect. However, if hemp is stored or dried incorrectly, moulds can form, which in turn can produce mycotoxins that can damage the liver and kidneys or even cause cancer. If the infested hemp is processed into tea, this can be harmful to the consumer.

Automated Clean-up with FREESTYLE SPE

Day, night and even on weekends - the automated FREESTYLE system takes care of your daily routine mycotoxin analysis tasks unattended around the clock, leaving you more time for other important laboratory tasks.

For the automated clean-up of aflatoxins and ochratoxins in hemp, extract, filter and dilute it according to the instructions of the processing protocol on the following page. Then place the sample in the FREESTYLE SPE, equip the racks with the necessary columns, parameterize the method in the software with a few mouse clicks and start the system - **ready**.

Matrix of the Month

Processing Protocol

Homogenize 10 g hemp and add 2 g sodium chloride. Subsequently extract the mixture with 100 mL methanol/water (80/20 (v/v)) and 50 mL n-hexane to remove essential oils and fats. To achieve high extraction efficiency, perform the extraction for 30 minutes.

Filter the raw extract and dilute 2 mL with 12 mL PBS (contains 8% Tween20). Then load 14 mL of the sample (equivalent to 0.2 g matrix) either on an AflaCLEAN column, AflaCLEAN Select column or OtaCLEAN column. To allow efficient binding of the toxins to the antibodies, the flow rate should not exceed 2 mL/min. Wash the column with 2 x 5 mL deionized water and use the washing solution to rinse the sample reservoir.

Elute the toxin with 2 mL methanol. Ensure that the methanol acts in the column bed for 5 minutes to ensure complete denaturation of the antibodies.

HPLC-Conditions

Aflatoxins B/G	
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))
Fluorescence detection:	Derivatization with UVE, Photochemical Reactor
Excitation wavelength:	365 nm
Emission wavelength:	460 nm

HPLC-Conditions

Ochratoxin A	
HPLC:	Isocratic
Column oven:	40 °C
Separation column:	RP EC 125/3 nucleosil 120-3 C18
Flow rate:	0.6 mL/min
Eluent:	HPLC-Water/Methanol/ Acetonitril (40/55/5 (v/v/v) + 1 % Acetic Acid)
Fluorescence detection:	without Derivatization
Excitation wavelength:	335 nm
Emission wavelength:	465 nm

Recovery Rates

Content of Aflatoxins B1, B2, G1, G2 and Ochratoxin A in Hemp

	Aflatoxins				OTA
Aflatoxin	**B1**	**B2**	**G1**	**G2**	
Standard*	100	100	100	100	100
Recovery Rate ** Hemp OtaCLEAN 20 ppb Ochratoxin A	-	-	-	-	102
Recovery Rate ** Hemp AflaCLEAN 20 ppb Aflatoxin B/G	103	101	102	100	-
Recovery Rate ** Hemp AflaCLEAN Select 20 ppb Aflatoxin B/G	101	101	101	100	-

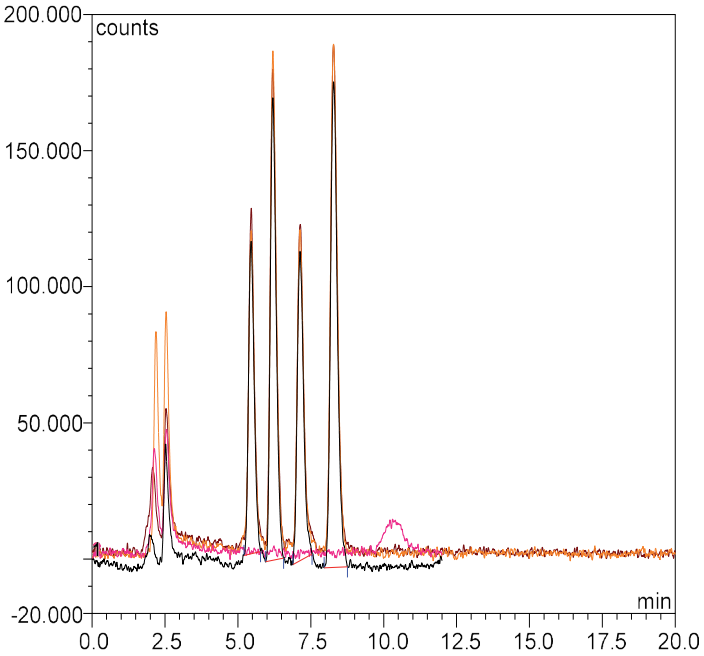
* Standard was set = 100% , ** Corrected with non-spiked sample / The results are in accordance with the performance specifications of the EC 401 / 2006 (section 4.3.1).

The recovery rates show an efficient enrichment of mycotoxins despite a difficult matrix, such as hemp and other medically relevant materials that are made storable by drying.

Chromatograms

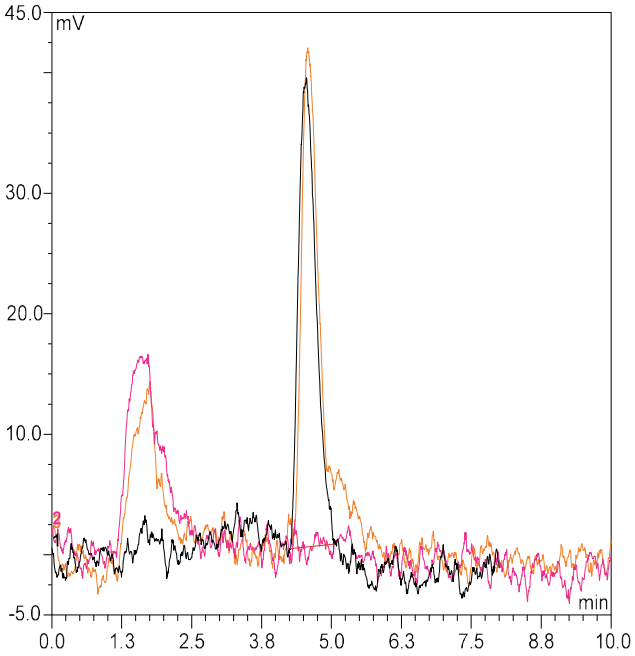
Matrix of the Month

Chromatogram Aflatoxins B/G



Black: Standard 4ng/2mL represents 20 ppb
Red: Hemp blind with AflaCLEAN
Orange: Hemp 20 ppb with AflaCLEAN
Brown: Hemp 20 ppb with AflaCLEAN Select

Chromatogram Ochratoxin A



Black: Standard 4ng/2mL represents 20 ppb
Red: Hemp blind with OtaCLEAN
Orange: Hemp 20 ppb with OtaCLEAN

LCTech Clean-up Columns

LCTech has developed immunoaffinity columns especially for the clean-up of mycotoxins in food and feed. Among others, AflaCLEAN, AflaCLEAN Select, AflaOtaCLEAN and OtaCLEAN for the clean-up of Aflatoxins B/G and Ochratoxin A.

All columns show a very high matrix tolerance, excellent clean-up and recoveries!

- ☑ The immunoaffinity columns are successfully used worldwide for various matrices in accredited laboratories.
- ☑ In international interlaboratory tests they have achieved excellent results.

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

All clean-up columns are suitable for automated processing, e.g. in the LCTech units, FREESTYLE SPE.

*Afla-OtaCLEAN for simultaneous clean-up of Aflatoxins B/G and Ochratoxins – **Save time & money!***

These LCTech Products were used:

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

AflaCLEAN Select Immunoaffinity Columns for Aflatoxin B/G
P/N 12058 / 12059

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

AflaOtaCLEAN Immunoaffinity Columns for Aflatoxin B/G and Ochratoxin A
P/N 11022 / 11771

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