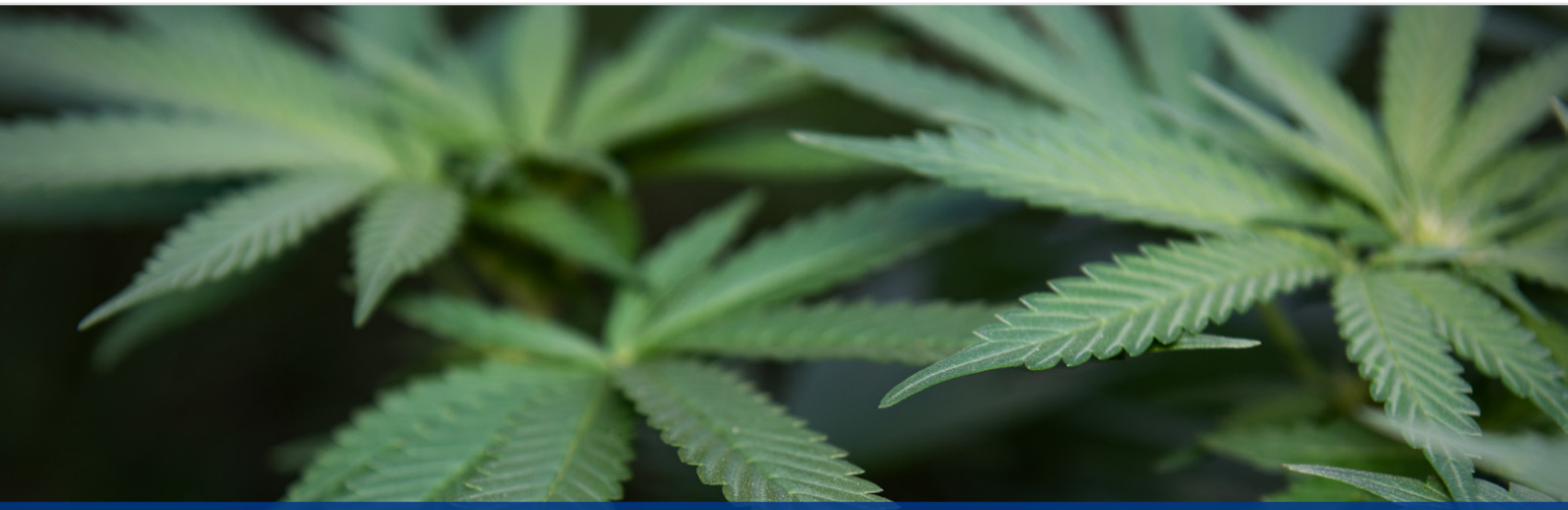
Matrix of the Month



May 2020

Aflatoxin B/G in Hemp
~ 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

For many laboratories, the challenge in everyday laboratory work is to analyze as many samples as possible in the shortest possible time, precisely and with maximum sensitivity. To make this task easier for you, LCTech has developed the AflaCLEAN immunoaffinity column as well as the AflaCLEAN SMART column.

Both columns are designed for the clean-up of aflatoxins B1, B2, G1 and G2 in food and feed, including hemp. The columns are able to bind the aflatoxins in a highly specific way and have a very high matrix tolerance.

By using the 3.5 cm SMART column, a particularly high sample throughput of up to 500 samples / week is achieved, since the processing time is significantly reduced due to the small size of the column. In addition, more than 80 % of solvents are saved during extraction, dilution, washing, sample introduction and elution.

Automated Mycotoxin Analysis with FREESTYLE ThermELUTE™

The robotic system FREESTYLE ThermELUTE™ in combination with SMART columns enables full automation with sensitive results that even in the lower ppt-range analysis can be performed around the clock, even at weekends.

The unique technology realizes a processing „from raw extract to chromatogram“ without any manual intermediate steps. Samples are always processed in the same way and you can be sure to fullfill European regulations and limits.

For the automated processing of hemp, extract, filter and dilute it according to the processing protocol on page 2. Then place the sample into the FREESTYLE, equip the racks with the necessary columns, parameterize the method in the software with a few clicks and press START - done.

Matrix of the Month

Manual Processing Protocol

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

Filter the raw extract and dilute 2 mL with 12 mL PBS (contains 8 % Tween20). Load 2.8 mL of the sample (corresponding to 0.04 g matrix equivalents) to an AflaCLEAN SMART column. For comparison purposes, an AflaCLEAN column was loaded in parallel.

To allow an efficient binding of the toxins to the antibodies, the flow rate of the AflaCLEAN SMART column should not exceed 3 mL/min and the flow rate of the AflaCLEAN column should not exceed 2 mL/min. Wash the SMART column with 2 mL of deionized water and use the washing solution to rinse the sample reservoir before washing the column.

Elute the toxins with 0.4 mL of methanol. Let the methanol act in the column bed for 5 minutes to ensure complete denaturation of the antibodies.

AflaCLEAN SMART and AflaCLEAN in Direct Comparison

Column type:	AflaCLEAN SMART	AflaCLEAN
Sample volume:	2.8 mL	14 mL
Flow rate:	3 mL/min	2 mL/min
Wash volume:	2 mL	10 mL
Elution volume:	0.4 mL	2 mL
Matrix equivalents / Eluate volume:	0.1 g/mL	0.1 g/mL
Time required:	< 10 min.	> 20 min.

Recovery Rates

Content of Aflatoxin B1, B2, G1 and G2 in Hemp

Aflatoxins B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate ** Hemp AflaCLEAN 20 ppb	100	92	93	84
Recovery Rate ** Hemp AflaCLEAN SMART 20 ppb (Error [n=3])	95 (+/- 1)	94 (+/- 5)	91 (+/- 4)	77 (+/- 3)

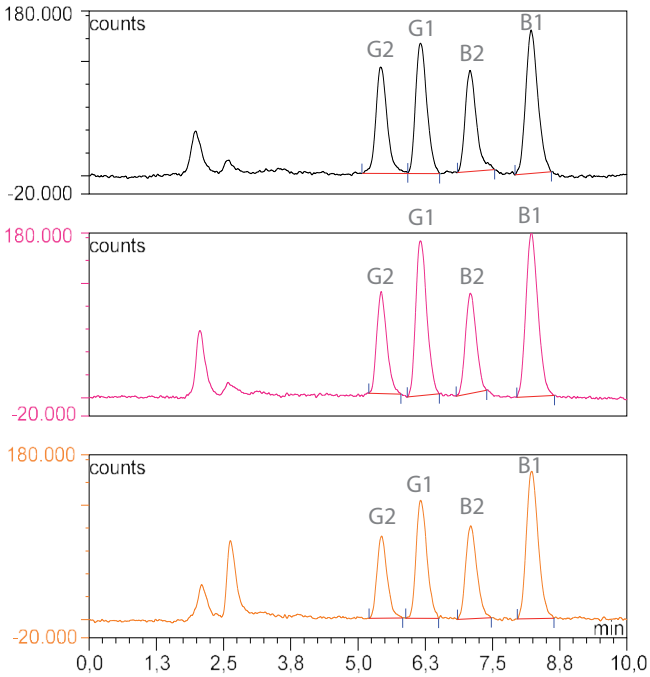
* Standard was set = 100% , ** Corrected with non-spiked sample / The results are in accordance with the performance specifications of the EC 401 / 2006 (section 4.3.1).

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

Matrix of the Month

Chromatogram Aflatoxine B/G

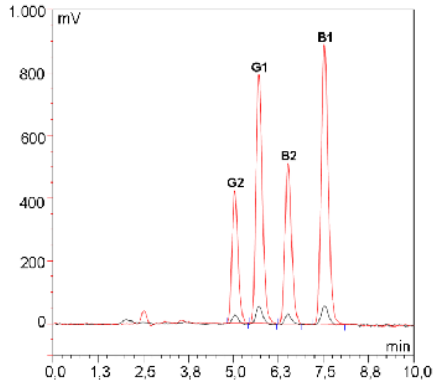


Black: Standard 4 ng/2 mL, represents 20 ppb Afaltoxins
Red: Hemp 20 ppb, cleaned-up with AflaCLEAN
Orange: Hemp 20 ppb, cleaned-up with AflaCLEAN SMART

Highest Sensitivity Through Automation

Full automation using the SMART columns in the FREESTYLE ThermELUTE™ enables not only a higher sample throughput but also the highest analytical sensitivity, since the eluate is quantitatively transferred into the HPLC system.

- Increase your sample throughput,
- increase your analytical sensitivity,
- and achieve reliable, reproducible results.



Red: 3 ppb sample (0.28 g matrix) directly injected through ThermELUTE™ technology

Black: 3 ppb matrix; column eluate diluted to HPLC conditions and injected

Sensitivity gain by a factor of 14

Learn more about FREESTYLE ThermELUTE™ at our YouTube Channel or on our Website.

Conclusion

The recovery rates show an efficient toxin enrichment, even for a difficult matrix like hemp. This demonstrate that both AflaCLEAN and its SMART version, with significantly lower solvent consumption, work with the same efficiency.

The chromatograms do not show any conspicuous interfering substances within 10 minutes, which allows a fast and accurate analysis of the samples. At the same time an analytical compatibility to the LC-MS/MS system is given.

Fast – Reproducible – Sensitive – SMART

Did You Know?

AflaCLEAN SMART and OtaCLEAN SMART can be combined for simultaneous clean-up of aflatoxins B/G and ochratoxin A. You receive a fast and maximum-specific clean-up.

There LCTech Products were Used:

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

AflaCLEAN SMART, Immunoaffinity Columns for Aflatoxin B/G
P/N 12862 / 12863

HPLC Separation Column RP C-18
P/N 10522

UVE Photochemical reactor
P/N 10519

FREESTYLE ThermELUTE™, Robotic System for Automated Sample Preparation and Analysis
P/N 12663 / 12668 / 13691