



# Aflatoxins in hazelnuts

Cleaned-up with **AflaCLEAN SMART** Manual und Automated



## Hazelnuts

The RASFF identified more than 240 complaints for nuts and nut products in 2021 that were due to mycotoxins. These mostly resulted in import bans or rejections at EU borders. Currently, nuts represent the largest fraction of food products rejected due to mycotoxins in the area of import at EU borders. Hazelnuts are frequently contaminated with aflatoxin G1/G2 but also B1.



AflaCLEAN SMART

## **AflaCLEAN SMART** - klein + schnell + günstig = SMART

The AflaCLEAN SMART columns are available in a small 3 cm polypropylene format, which has significant handling and storage advantages over larger columns.

### The advantages at a Glance:

- Size: only 3 cm
- Solvent saving of 80 % due to miniaturisation
- Shelf life: 9 months cooled at 4 to 8 °C
- Loading capacity: 100 ng aflatoxin B1
- Recoveries:  
B1 > 90 %, B2 > 80 %, G1 > 90 %, G2 > 60 %
- Suitable for manual and automated processing

The processing time for extraction, dilution, washing and elution is significantly reduced. For example, no column emptying is necessary and loading the sample and wash-

ing the column only takes a maximum of 4 minutes with a 10 mL sample feed and a flow rate of 3 mL/min. Since a maximum of only 400 µL is also used for elution, this reduces the processing time even further. The functional mechanism is based on the principle of immunoaffinity chromatography with increased flow rates and an adapted, low elution volume to save processing time. The material in the column is coated with antibodies directed against aflatoxin B and G. The sample extract is loaded onto the column. When the sample extract is loaded, the aflatoxins B and G are retained in the column while the matrix components pass through the column. After a washing step, the aflatoxins B and G can be quantitatively eluted from the column by methanol and subsequently analysed by HPLC.

## Manual processing with AflaCLEAN

Extract 20 g of homogenized hazelnut with 100 mL of 80/20 (v/v) methanol/water. Add 2 g sodium chloride and 50 mL n-hexane during extraction to separate fats and oils. After stirring for 10 min, filter the crude extract and optionally centrifuge at 3000 x g to ensure efficient phase separation between the methanolic extract and the n-hexane. Now dilute 3.5 mL of the n-hexane

free extract with 21.5 mL of PBS buffer. Load 10 mL of this diluted sample onto the AflaCLEAN SMART column at 3 mL/min. Then rinse the sample tube with 2 mL of deionized water and load this onto the column as well. After drying the column, elute the toxin through 400 µL methanol and measure an aliquot after dilution to run ratios in HPLC using fluorescence after photochemical derivatization by the UVE.



## Automation with the **FREESTYLE ThermELUTE™** robotic system

The sample clean-up process can also be fully automated on a FREESTYLE system with ThermELUTE™ module. Parameterization is performed via the software, the sample list is generated, and the sample introduction vessels and columns are placed in the instrument. After less than 30 minutes, the first result can be assessed using the chromatography software. After this first cycle, another result follows every 20 minutes, so a throughput of up to 120 samples in 24 hours can be achieved. Automation with the FREESTYLE ThermELUTE™ robotic system enables high sample throughput (500 samples/week). The system can be combined with a direct injection module with any HPLC or LC-MS system - the result is comprehensive automation with processing from raw extract to finished chromatogram without manual steps with convincing advantages:

- Sample processing day and night
- Reproducible results
- Excellent recovery rates
- Remarkable sensitivity in the lower ppt range  
→ Every matrix below EU limits, even in baby food

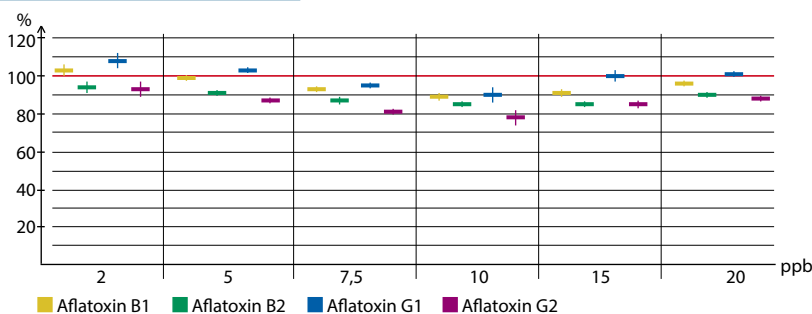
Conditions	
HPLC / UPLC	Isocratic
Column oven	36 °C
separation column	#10522 RP C-18
Flowrate, Solvent	1.2 mL/min; running medium water/methanol/acetonitrile (60/30/15 (v/v/v))
Fluorescence detection with photochemical post-column derivatization	Derivatization by UVE (photochemical) (PN 10519)
Excitation wavelength	365 nm
Emmission wavelength	460 nm

Recovery rates** in hazelnuts				
Aflatoxin	B1	B2	G1	G2
Standard*	100	100	100	100
2 ppb	103	94	106	93
5 ppb	99	91	103	87
7.5 ppb	93	87	95	81
10 ppb	89	85	90	78
15 ppb	91	85	100	85
20 ppb	96	90	101	88

\* Standard was set = 100%

\*\* Corrected with non-spiked sample / The results are in accordance with the performance specifications of EC 401 / 2006 (section 4.3.1). The recovery rates are mean value.

## Recovery rates



Automated clean-up with the FREESTYLE ThermELUTE™ robotic system

## Conclusion

Consistent recoveries in the range of 2 - 20 ppb (total toxin). Recoveries are reproducible, fast, efficient and samples can be analyzed fully automated with good chromatographic performance.

### These LCTech products were used:

10522 Mycotoxin HPLC-column  
 10519 UVE  
 AflaCLEAN SMART 12863 (1000 pc/PU);  
 10862 (100 pc/PU)

Do you have a special request as to which matrix we should test for you?  
 Contact us by e-mail at: [info@LCTech.de](mailto:info@LCTech.de)