



Aflatoxin B/G and Ochratoxin A in Wheat

Cleaned-up with *AflaCLEAN* and *OtaCLEAN*



Wheat in food / feeding stuff

Wheat is, beside rice and corn, the most often used cereal in foodstuff (bakery products / pasta) or feeding stuff. Seasonal harvesting as well as storage in large quantities or excessive humidity during storage, favor the growth of molds and the formation of mycotoxins. The high prevalence and toxicity require a special sample analysis not only for food but also for baby food, as the permissible limits are much lower than for food or feed stuff.

By November 2022, the amount of positive and non-approved grain detected during border inspections for mycotoxin prevalence had more than doubled in one year. (RASFF Portal online database).

Sensitive analysis of *Aflatoxin B/G* and *Ochratoxin A* in wheat

3 mL immunoaffinity columns AflaCLEAN or OtaCLEAN:

- **High matrix loading** and **analyte enrichment** for optimal determination of analytes in the trace analysis (baby food)
- Maximize the value of your analytical benefit through **compatibility** with **LC-MS/MS** and **HPLC fluorescence**
- **High loading capacities, excellent recoveries** over a wide measuring range (baby food - feed)

Processing protocol

20 gram homogenised cereals are added to 100 mL (80/20 (v/v)) methanol/water. The extraction takes place for at least 5 minutes using an ultraturrax or blender jar to achieve best extraction efficiency. The extract is filtered through a plaited filter to remove particles.

Dilute the crude extract (10.5 mL) with 64.5 mL pbs buffer. Filter the diluted sample by a whatman GF/A filter to remove turbidity.

Apply a maximum of 50 mL (representing 1.4 gram matrix equivalents) on the AflaCLEAN or OtaCLEAN column, with a maximum flow rate of 2 mL/min. Wash the sample reservoir and the AflaCLEAN or OtaCLEAN column stepwise with a total of 10 mL water. Remove residual water by flushing air through the column.

Elute the toxins by incubating the column bed for 5 minutes with methanol, the eluate is collected in a 2 mL measuring cylinder. After thoroughly mixing the eluate HPLC or LC-MS/MS analysis could be prepared.



3 mL + SMART AflaCLEAN, OtaCLEAN and Afla-OtaCLEAN columns

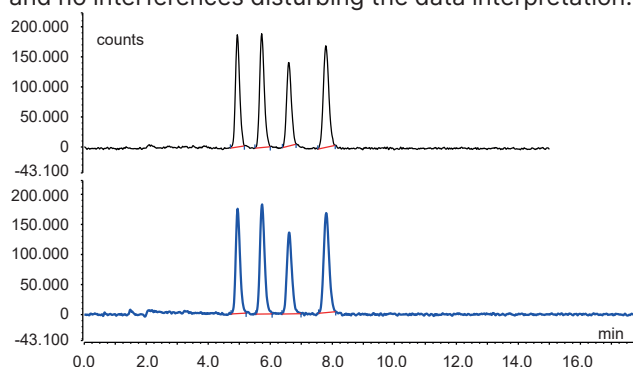


Recovery rates for Aflatoxin B/G and Ochratoxin A

The specific clean-up reduces matrix interferences and concentrates the analytes for excellent analysis of cereal on **food and baby food level**. The spiking experiments (4 ppb aflatoxin B1/G1 and 1 ppb aflatoxin B2/G2) and 5 ppb Ochratoxin A revealed excellent recoveries. The chromatographical clearance demonstrates compatibility with LC-MS/MS and HPLC fluorescence analysis. Recoveries from 96 to 98 % for all analytes could be found.

Chromatogram

The chromatographic results for Aflatoxins revealed no matrix interferences and allows the analysis of all 4 Aflatoxins under the mentioned chromatographical conditions. A maximum chromatography time of 10 minutes was chosen, and revealed good peak separation and no interferences disturbing the data interpretation.



Chromatographic results for aflatoxin B/G.

Black: Chromatogram of an aflatoxin standard
Blue: Chromatogram of a cleaned wheat sample (spiked with aflatoxin before extraction)

Conclusion

The clean-up of wheat and cereals using the immuno-affinity clean-up cartridges AflaCLEAN or OtaCLEAN is suitable for Aflatoxin B/G or Ochratoxin A analysis over a wide range of contamination level, covering feeding stuff to baby food with best recoveries, matrix depletion and could be used for analysis either by HPLC fluorescence or LC-MS/MS analysis with reliable and best results.

Recovery rates**

Aflatoxin B1 (4 ppb)	96 %
Aflatoxin B1 (0.1 ppb)	98 %
Aflatoxin B2 (1 ppb)	96 %
Aflatoxin B2 (0.025 ppb)	98 %
Aflatoxin G1 (4 ppb)	96 %
Aflatoxin G1 (0.1 ppb)	96 %
Aflatoxin G2 (1 ppb)	98 %
Aflatoxin G2 (0.025 ppb)	95 %
Ochratoxin A (10 ppb)	98 %
Ochratoxin A (0.5 ppb)	98 %

** Corrected with non-spiked sample / The results meet the performance criteria according to EC 406/2001.

LC-Conditions

	Aflatoxin B/G	Ochratoxin A
Solvent (water/methanol/acetonitrile)	60/30/15	40/55/5 +1% acetic acid
Flowrate (mL/min)	1.2	1.0
Column	PN 10522	PN 10522
Column temperature	36 °C	40 °C
Fluorescence Ex.	365 nm	335 nm
Fluorescence Em.	460 nm	465 nm
Post column derivatisation	UVE	No
Retention time (min)	4.95 (G2) 5.73 (G1) 6.6 (B2) 7.8 (B1)	6.9 (OTA)

These LCTech products were used:

10514	AflaCLEAN
10515	OtaCLEAN
10522	Mycotoxin HPLC-column
10523	Guard column
10750	Guard column holder
10519	UVE (photochemical derivatisation of aflatoxins)

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