Aflatoxin B/G and ochratoxin A in dog and cat food

Clean-up with *Afla-OtaCLEAN*

Dog and cat food

In 2020, the USFDA published a list of feed lots that contained excessive levels of aflatoxin. As a result of the use of these feeds, several hundred animals were harmed. In most cases, high grain contents are responsible for the high aflatoxin levels. Also in 2021, similar findings were linked to illness in several hundred cats in the UK.

The quality and testing of feed is essential to provide a high quality, healthy feed and is even more difficult the more complex the composition of the feed. The composition of the ingredients poses high challenges for sample preparation in the field of mycotoxin analysis, which can be harmful to the health of the animals even in small doses.

Afla-OtaCLEAN - best results even with difficult matrices

The Afla-OtaCLEAN columns are available in the convenient 3 mL polypropylene format, which has clear advantages in handling compared to, in relation to the column bed, smaller diameters. The Afla-OtaCLEAN immunoaffinity column is designed for sample preparation within food analysis using HPLC with fluorescence detection or LC-MS. LCTech offers the perfect solution to reduce your working time during clean-up. Analyse your sample for multiple mycotoxins in only one step.

Advantages at a glance:

- 3 mL format
- Shelf life: 18 months at room temperature between 4 and 30° C
- Capacity Aflatoxin B1: 150 ng Aflatoxin B1
- Capacity Ochratoxin A: 200 ng Ochratoxin A
- Best recoveries: B1 > 90 %, B2 > 80 %, G1 > 90 %, G2 > 60 %, ochratoxin A > 90 %
- Suitable for automated processing

Immunoafinity columns Afla-OtaCLEAN

LCTech

Processing Protocol

Weigh out 20 g of homogenised feed and add 2 g of sodium chloride to this matrix.

Extract the toxins by adding 100 mL methanol/water (80/20(v/v)) and 50 mL N-hexane. The extraction is optimally done for 3-5 minutes in an Ultraturrax (blender jar) or by stirring or shaking for at least 30 minutes at room temperature. Filter the extract and centrifuge the filtrate at 3000 xg for 5 minutes. For further processing use the lower n-hexane free phase.

Dilute 2 mL of the n-hexane free phase with 12 mL of PBS buffer (8% Tween 20) and mix the sample carefully.

Load the diluted sample (14 mL corresponds to 0.4 grams of matrix) onto the Afla-OtaCLEAN immunoaffinity column at a maximum flow rate of 1-2 mL/min. Then the sample introduction vessel is rinsed twice with 5 mL deionised water and the rinsing solution is also loaded onto the column. Before washing the column, make sure that no sample residues remain above the column bed, otherwise the washing efficiency will deteriorate.

After washing, dry the column with a short stream of air. When the column is completely dry, elute the toxin with 2 × 1 mL methanol. It is important to incubate the methanol for 5 minutes in the column bed to achieve complete denaturation of the antibodies. Eluate residues are also removed from the column with slight overpressure and collected together with the eluate. Finally, the eluates are diluted/adjusted to running medium ratios and measured by liquid chromatography, fluorescence detection or LC-MS/MS.

Tip from the expert

By using photochemical derivatisation (UVE), the fluorescence intensities of toxins B1 and G1 are increased more than tenfold. This allows a more precise, faster analysis without the use of further derivatisation reagents. The compatibility of photochemical derivatisation with HPLC, but also with UPLC, is given and enables fast, baseline-separated chromatography of all aflatoxins and a reduction of interfering substances and halogenated waste.

Conditions	
HPLC / UPLC	isocratic
Separation column	36 °C
Trennsäule	P/N 10522 RP C18 150 mm
Flowrate, Solvent	1.2 mL/min; running medium water/Methanol/Acetonitril (60/30/15 (v/v/v))
Fluorescence detection	With derivatisation (UVE/photochemical)
Excitation wavelength	365 nm
Emission wavelength	460 nm

Recovery rates**					
Toxin	B1	B2	G1	G2	OTA
Standard*	100	100	100	100	100
dog food 20 ppb	93	93	92	93	97
cat food 20 ppb	85	87	86	87	99

* 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).

Conclusion

The analysis of complex animal feeds by means of Afla-OtaCLEAN allows fast and valid testing for the mould toxins aflatoxin B/G and ochratoxin A, compatible with all liquid chromatographic analysis methods, whether LC-MS/MS, HPLC-FLD or UPLC-FLD. With highly selective clean-up columns, fast, precise analyses and a high sample throughput are made possible by short chromatography times.

These *LCTech products* were used:

11022 Afla-OtaCLEAN (25 pcs/pack)
10522 HPLC column for mycotoxins
10519 UVE - photochemical device for aflatoxin derivatisation

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

LCTech GmbH
Daimlerstr. 4
84419 Obertaufkirchen, Germany
Tel. +49 8082 2717-0
info@LCTech.de
www.LCTech.de

February 2022