Mycotoxins in dog and cat food

Cleaned-up with *CrossTOX®*

Dog and cat food

In February we reported on the high levels of aflatoxins in dog and cat food that were objected by the USFDA. High contamination rates with increased occurrence of mycotoxins are not uncommon in feed. This requests for explicit sample preparation before analysis. Especially protein- and fat-containing matrices create difficulties in analysis if no appropriate sample clean-up is done. It is advisable to carry out a depletion of the impurities in order to be able to examine clean samples with fewer interfering substances.

By using the CrossTOX® column, you enable highly efficient sample clean-up even for complex matrix composition to analyse 17 mycotoxins simultaneously, while a quantification of most of them is even possible without addition of internal standard. Due to its non-dispersive SPE material, it allows optimal separation of matrix interferences before the analysis by LC-MS/MS takes place.

CrossTOX® – 17 mycotoxins at one go!

The CrossTOX® columns by LCTech allow a high efficient sample clean-up of all regulated plus expected mycotoxins. At the same time, the columns improve the conventional dilute-and-shoot process via a QuEChERS based procedure:

- ***High purity*** of extracts due to special sorbents for high matrix binding; no separate filtration
- Significantly ***less internal standard*** required due to selective matrix retention not influencing analytes of the columns
- ***Less cleaning and maintanance*** of your LC-MS/MS system due to the high purity of the extracts
- ***Only one column*** for a wide range of matrices

Catch them all

Aflatoxine B1, B2, G1, G2
Ochratoxin A
Zearalenon
Deoxynivalenol
Fumonisine B1, B2
T-2
HT-2
Nivalenol
3-Acetyl-DON
15-Acetyl-DON
DON-3Glc
Sterigmatocystin
Citrinin
Diacetoxyscirpenol

CrossTOX®

Processing protocol

Extract 20 g homogenised cat or dog food by adding 100 mL extraction solution (84 % acetonitrile / 15 % water / 1 % acetic acid (v/v/v)). Extract for at least 10 to 30 minutes, depending on the extraction device.

Filter the extract or sediment the insoluble components by centrifugation. Add 1 to 3 mL of the liquid extract to the CrossTOX® column at a flow rate of 1 - 2 mL / min. The flow through fraction can be measured directly by LC-MS/MS under the necessary instrument settings for the mycotoxins.

LCTech

CrossTOX® comparison

The CrossTOX® columns are suitable for both manual and automated processing. In the following illustration you will find your advantages compared to the conventional dilute-and-shoot method:

	Dilute-and-Shoot	CrossTOX® Manual processing	CrossTOX® Automated processing with FREESTYLE
Extraction	30 – 90 minutes	5-10 minutes	5-10 minutes
13C Internal Standard	For all Toxins needed	For less toxins needed	For less toxins needed
Dilution of Extract	Necessary	Not necessary	Not necessary
Way of Filtration	Syringe filtration	Column filtration	Not necessary
Dispensing	In vial	In vial	Direct injection to LC-MS/MS
Cleanliness of Extract	Not clean enough	Very clean	Very clean
LC-MS/MS Maintenance	Permanent maintenance	Less maintenance	Less workload
Process time / Manpower	Heavy workload	Heavy workload	Less workload

Conclusion

The CrossTOX® columns can be used to process simple matrices such as cereals, dried fruits and nuts, but also more complex samples such as feed and spices can be made easily accessible for LC-MS/MS analysis without matrix interference affecting or contaminating the analysis.

In addition, a higher sample throughput can be achieved without drift in the analysis or a need for matrix calibration/match. Especially in the field of feed analysis, where many samples are often contaminated with more than one toxin, an efficient, fast and instrument-friendly analysis is an advantage. You retain the full measurement sensitivity of your LC-MS/MS and costs for internal standards can be easily saved.

This LCTech product was used:

17900 CrossTOX® 100 pieces/pack

Recovery rates				
Analyt	**Toxin level (ppb/ µg/ kg)**	**Dog food**	**Cat food**	**Internal standard**
Aflatoxin B1	8	86	88	n.n.
Aflatoxin B2	2	96	103	n.n.
Aflatoxin G1	8	89	87	n.n.
Aflatoxin G2	2	84	87	n.n.
Ochratoxin A	10	86	89	n.n.
Zearalenon	200	86	88	n.n.
Fumonisin B1	2500	102	100	n.n.
Fumonisin B2	2500	100	102	n.n.
Deoxynivalenol	2000	102	99	n.n.
Nivalenol	2000	84	77	n.n.
Sterigmatocystin	20	84	94	n.n.
T2	50	98	97	rec
H-T2	50	88	94	rec

* n.n. (not necessary)
** rec (recommended)

Conditions	
UPLC	gradient
Column oven	40 °C
Separation column	Accucore Biphenyl 100 mm x 2,1 mm; 2.6 µm with precolumn
Flowrate, Solvent	0.4 mL/min; Solvent A: HPLC water/methanol (98/2 (v/v), 5 mM ammonium acetate, 1% acetic acid) Solvent B: HPLC water/methanol (2/98 (v/v), 5 mM ammonium acetate, 1% acetic acid)
0 - 2 min	95 % A; 5 % B
2 - 5 min	15 % A; 85 % B
5 - 11 min	5 % A, 95 % B
11 - 16 min	95 % A; 5 % B
Analytics	Heated ESI 3500 V (+); 1500 V (-); Ion transfer tube 325°C; Evaporator 350 °C.

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

LCTech GmbH
Daimlerstr. 4

84419 Obertaufkirchen, Germany
Tel. +49 8082 2717-0

info@LCTech.de
www.LCTech.de