Aflatoxins and Ochratoxin A in marzipan and drinking cocoa

Manual and automated clean-up for *LC-MS/MS analysis*

Marzipan and drinking cocoa

One thing can not be missed at Christmas time: Treats, such as marzipan or a hot chocolate! At this time of year, hardly anyone can resist these sweets. But as delicious as it tastes - it can be dangerous. Because the two end products are processed from almonds and cocoa beans, which are mainly imported from third countries. A long and wrong storage makes them more susceptible to molds that produce highly toxic, aflatoxins and ochratoxin A. In order to be able to control excessive toxin contamination, especially during importation, the European Commission's implementing regulation has set limits for these mycotoxins that may not be exceeded.

18 mycotoxins at once - *CrossTOX®* makes it possible

LCTech's CrossTOX® columns enable highly efficient sample purification of regulated and expected mycotoxins. At the same time, they improve the conventional dilute-and-shoot application by a QuEChERS-based non-dispersive (SPE) purification procedure.

A specially designed sorbent guarantees a high removal of analytically interfering sustances even from difficult matrices. CrossTOX® can be used to purify both cereal-based matrices as well as nuts, dried fruits and spices can be processed with excellent purification of contaminants with very good results. The loading capacity is 3 mL (corresponds to 0.6 g matrix).
Purification via CrossTOX® is either manual or automated possible with a FREESTYLE SPE robotic system or even fully automated in combination with an HPLC Direct Injection module. Depending on the matrix, the majority of analytes are measured without internal standards and with excellent chromatographical results and recoveries. Internal standards and increasing sample purity, also decreases the enormous costs per sample as well as maintenance costs and chromatography times (LC-MS/MS).

Manual or automated clean-up
with the CrossTOX®:

Video

Processing protocol

Extract 20 g of matrix with 100 mL (84/16 (v/v)) Acetonitrile/Water. After stirring for 30 min, centrifuge the crude extract at 3000 x g and transfer up to 3 mL of the supernatant through the CrossTOX® column into a sample vial using a syringe. An aliquot is analyzed for the mycotoxins aflatoxin B/G and ochratoxin A by LC-MS/MS.
In this way, many samples can be quickly prepared for LC-MS/MS analysis by means of SPE without toxins remaining in the filter material or interfering substances from the matrix, such as fats and carbohydrates, colorants and aromas, negatively influencing the analysis.

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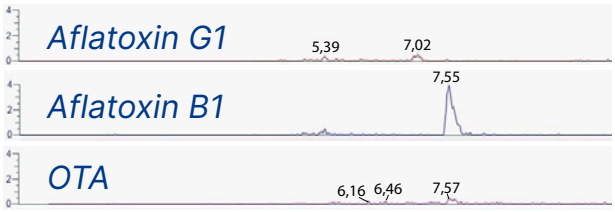
Chromatograms



Traces of aflatoxin G1 and aflatoxin B1 were found in the un-spiked matrix. Traces of OTA could also be detected. Qualitative differences between the marzipan samples were only met on a mycotoxin basis.
Comparative analysis of the matrix marzipan using AflaCLEAN and CrossTOX® Clean-up:

Aflatoxin	B1	B2	G1	G2
Clean-up	[µg/Kg]	[µg/Kg]	[µg/Kg]	[µg/Kg]
CrossTOX®	7.44	0.66	1.04	<0.5
AflaCLEAN	7.3	0.84	1.95	0.15

Conclusion

The CrossTOX® column not only impresses with good recovery rates, but also shows its strength in reliable quantification without using internal standards, thus enabling immense cost savings per sample. Further advantages are no contamination of injector, cone sweep or transfer tube. This processing protocol allows high sample throughput, significantly extended maintenance intervals and cost reduction. The results obtained show an equally good performance as the immunoaffinity columns (see table above).

These LCTech products were used:

17900	CrossTOX®
10514	AflaCLEAN
10519	UVE
10522	Mycotoxin HPLC column

Recovery rates**

Aflatoxin	B1	B2	G1	G2
Standard*	100	100	100	100
marzipan 20 ppb	93	80	94	86
drinking cocoa 20 ppb	92	10	92	84
Ochratoxin		A		
Standard*		100		
marzipan 20 ppb		103		
drinking cocoa 20 ppb		87		

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

Conditions

UPLC	gradient
Column oven	40 °C
Separation column	Accucore Biphenyl 100 mm × 2.1 mm; 2.6 µm with precolumn
Flow rate, Solvent	0.4 mL/min; eluent A: HPLC-water/methanol (98/2 (v/v), 5 mM ammonium acetate, 1 % acetic acid) eluent 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; Vaporizer 350 °C

Analytes and considered product ions

m/z	precursor	product ions
Aflatoxin B1	313.06	**285.11** / 213.05 /241.07 / 269.07 / 270.04
Aflatoxin B2	315.06	**287.05** / 259.05 / 271.02 / 243.05
Aflatoxin G1	329.10	**243.07** / 200.07 / 215.14 / 283.10
Aflatoxin G2	331.05	**245.10** / 189.10 / 275.03 / 285.06 / 313.11
Ochratoxin A	404.04	**238.97** / 221.04 / 341.04 / 358.05

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

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