

SOLUTIONS BY



Multi-Mycotoxin Analysis CrossTOX® Sample Clean-up and Matrix Removal

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1. Introduction

In the last years increasing numbers of mycotoxin contamination in food and feed were reported. Furthermore not only single mycotoxins but up to 90 % of all sample material contains more than one mycotoxin. With this the multi-mycotoxin analysis becomes more relevant. To overcome the workload issue with increasing sample numbers and to remain in compliance with regulated levels of mycotoxins.

The matrices which are investigated for mycotoxins are not only cereals or cereal based anymore, but more food and feed stuff that contains nuts or peanut with high fat components or contain oils are investigated. These disturb the analytical devices and lead to implementation of more and more complicated extraction procedures. Dried fruits as another matrix group is described in literature to be affected by toxic mycotoxins as well.

The CrossTOX® column allows the analysis of at least 18 mycotoxins extracted from one sample with only one extraction protocol for most matrices and an impressive matrix removal. CrossTOX® is the next step to common protocols for mycotoxin sample clean-up for multi mycotoxin analysis in the LC-MS/MS environment. The column clean-up is not restricted to only one type of matrix, but allows the mycotoxin clean-up and matrix removal from cereals, dried fruits or nut matrices as well as some spices could be processed with the CrossTOX® column. This leads to cost reduction and decreases the downtime of the analytical devices due to higher clearance of the sample with lower matrix interferences.



2. Method Development

2.1 Reagents and Materials

- CrossTOX® column (P/N 17900)
- UPLC-MS/MS H-ESI,
- Accucore Biphenyl 100 mm x 2.1mm; 2.6 µm with defender Guard column
- Labmill or equivalent
- Blender jar
- Syringe (10 mL), single use
- Beaker
- Laboratory balance (+/- 0.1 gram)
- Pipettes (0.2 – 1.0 mL)
- Optional plaited filter or centrifuge (3000xg)
- GC –vials with cap
 - Acetonitril p.a.
 - Water hplc grade
 - Acetic acid p.a.
 - Extraction solvent: Acetonitrile /water/acetic acid (84/15/1 (v/v/v))
 - n-hexane

2.2 Material for Automated Clean-up with Direct Injection

2.2.1 Configuration FREESTYLE

1. FREESTYLE BASIC	P/N 12663-12
2. FREESTYLE SPE module	P/N 12668
3. QuEChERS Set (Hardware and Software)	P/N 16269
4. HPLC Direct-Injection module	P/N 16278
5. Sample loop, stainless steel, 10 µL	P/N 14486
6. Relay cable assembly for AGILENT-system	P/N 13679
7. Rack for solvent delivery	P/N 13156
8. Overflow Sensor for Waste Level Control	P/N 12709
9. Frame for trays	P/N 11915
10. Rack for 60 SMART columns	P/N 13497
11. Adapter/Outlet for SPE-columns	P/N 13544
12. Frame for trays	P/N 11915
13. Tray for 60 pcs - 1 mL GC vials	P/N 11920
14. Tray for 60 pcs - 4 mL vials	P/N 11926
15. Tray for 30 pcs - 16 mL vials	P/N 11933
16. Retention plate for 1mL and 4 mL rack	P/N 13429

2.3 Sample Preparation for Cereal, Nut or Dried Fruit Matrices

20 g of sample material for cereals, nuts or dried fruits are homogenized and weight in. 100 mL of extraction solvent are added. The sample is extracted in a blender jar to ensure highest extraction efficiency. Check extraction time in your laboratory (according to experience the extraction time is 5 - 10 min). Let the sample settle for a few seconds. The supernatant could be used immediately. To avoid backpressure in the CrossTOX® column, an optional filtration through a plaited filter or centrifugation could be used for clearance of flour samples. The CrossTOX® column will trap smaller particles efficiently and remove those from the sample. A reduction of sample volume could help to overcome the backpressure in the column.

2.4 Sample Preparation for Spices

10 g of sample material for spices are homogenized and weight in. 100 mL of extraction solvent are added. Add 50 mL of n-hexane to remove fats and oils from the raw material during the extraction procedure. The sample is extracted in a blender jar to ensure highest extraction efficiency. Check extraction time in your laboratory (according to experience the extraction time is 5 - 10 minutes). Let the sample settle approx. one minute. The n-hexane free (lower) phase could be used immediately. A centrifugation could help to improve phase separation. Use the lower solvent phase (n-hexane free) for the further sample clean-up.

The sample is passed through the CrossTOX® column. Depending on the sample, impurities and dyes (0.5 - 3 mL) could be passed through the column. The filtrate is collected in the GC-vial and analysed in the LC-MS/MS for multi-mycotoxin status. Reduced sample volume increase matrix clearance, volumes down to 0.25 mL could be applied on the CrossTOX® column.

2.5 Instrumentation

UPLC column Accucore Biphenyl 100 mm x 2.1 mm; 2.6 µm with defender Guard was used. The sample is passed through the CrossTOX® column. Depending on the sample, impurities and dyes (0.5 - 3 mL) could be passed through the column. The filtrate is collected in the GC-vial and analysed in the LC-MS/MS for multi-mycotoxin status.

Column temperature was set to 38 °C. Eluent a): HPLC water/methanol (98 / 2 (v/v)) + 1 % acetic acid + 5 mM ammonium acetate. Eluent b): HPLC water/methanol (2 / 98 (v/v)) + 1 % acetic acid + 5 mM ammonium acetate. Flow rate 0.4 mL / min.

Table 1: UPLC gradient

Time [min]	Eluent A (%)	Eluent B (%)	Curve
0 - 2	95	5	5
2 - 5	15	85	5
5 - 11	5	95	5
11 - 13	95	5	5
13 - 16	95	5	5

MS settings: heated ESI, 3500 V positive / 1500 V negative ion voltage. Ion transfer tube temperature 325 °C, vaporizer temperature 350 °C.

For all analytes at least one quantifier and two qualifier product ions were chosen.

The matrices were spiked prior and after extraction to investigate extraction efficiency and matrix interferences. The spiking was performed using a broad range of toxin contamination levels ranging from 2 - 20 ppb for aflatoxins and 10 - 50 ppb for ochratoxin A and sterigmatocystin 15 - 50 ppb for T2 /H-T2 toxin and 50 - 500 ppb for zearalenone. Deoxynivalenol, derivatives thereof and nivalenol were investigated from 200 - 1000 ppb.

3. Results

Chromatographic separation

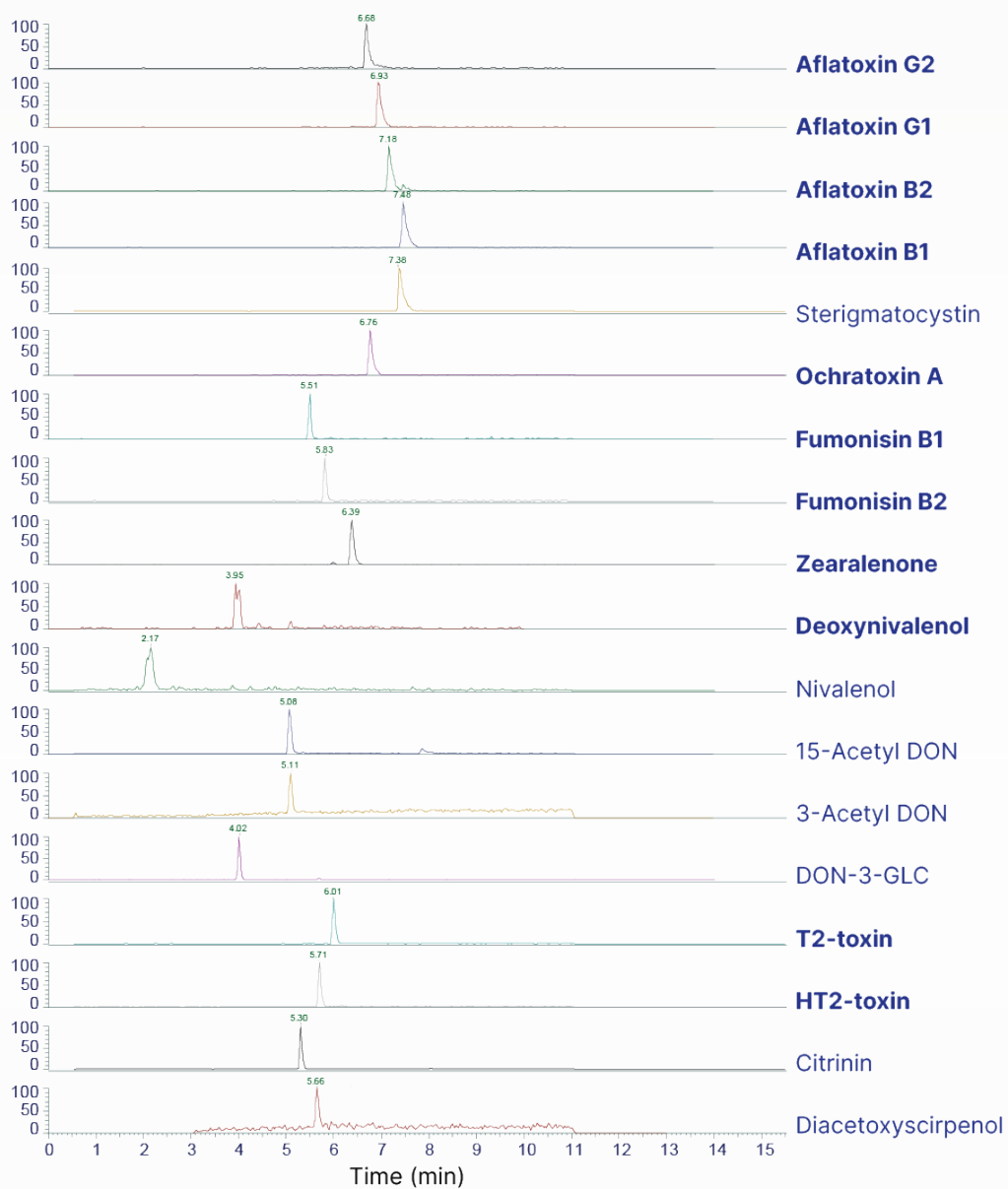


Figure 1: Chromatographic appearance for individual toxins using CrossTOX[®] clean-up procedure (regulated mycotoxins are marked in bold)

3.1 Cereal Matrices

Table 2: Results for cereal based matrices (the abbreviations used in the table correspond in the order in which the diagrams are labelled)

	Afla B1	Afla G1	Afla B2	Afla G2	OTA	ZEA	DON	F-B1	F-B2	T2-Toxin	HT2-Toxin	NIV	3-Ac-DON	15-Ac-DON	DON 3-Glc	STC
Spelt	95	79	95	113	84	81	94	116	116	74	79	94	88	73	94	83
Rice	102	100	127	76	107	98	72	113	120	99	93	105	104	113	82	119
Wheat	100	98	114	73	100	87	104	103	111	81	77	103	102	90	78	112
Oats	110	91	84	88	108	85	93	109	105	100	78	91	87	77	77	116
Corn	108	109	84	93	104	84	104	115	115	110	84	86	97	80	78	112

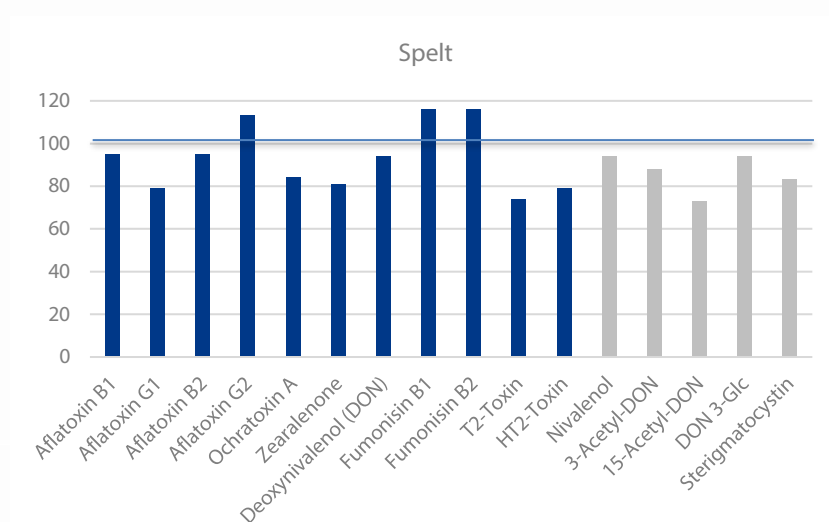


Figure 2: Recovery rates for Spelt

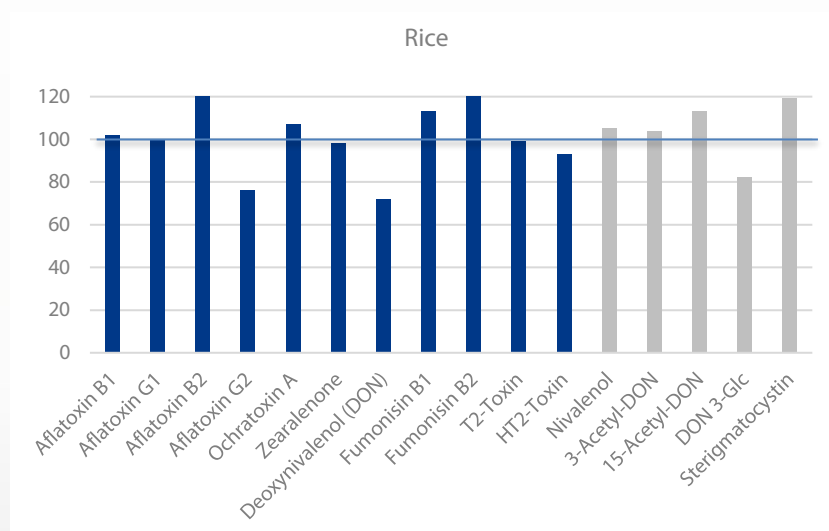


Figure 3: Recovery rates for Rice

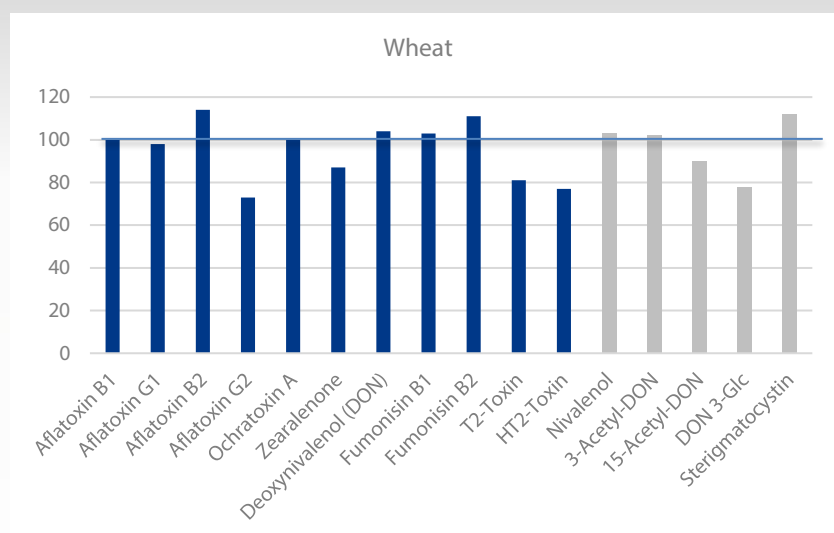


Figure 4: Recovery rates for Wheat

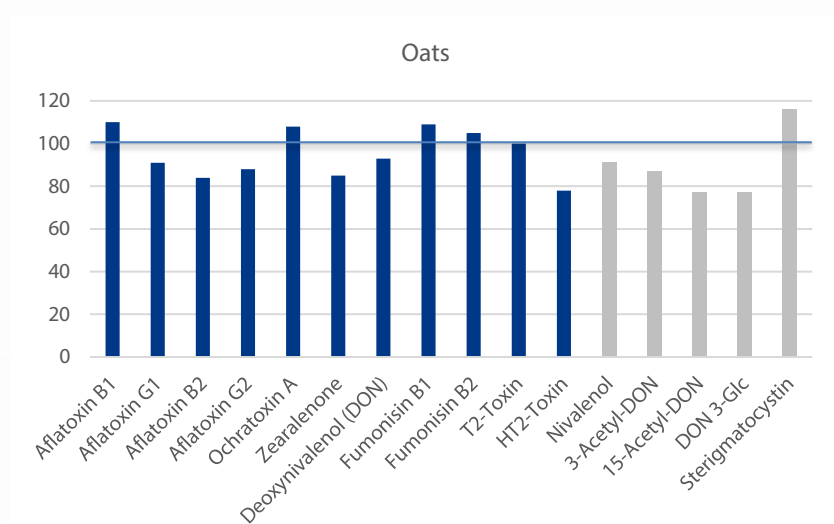


Figure 5: Recovery rates for Oats

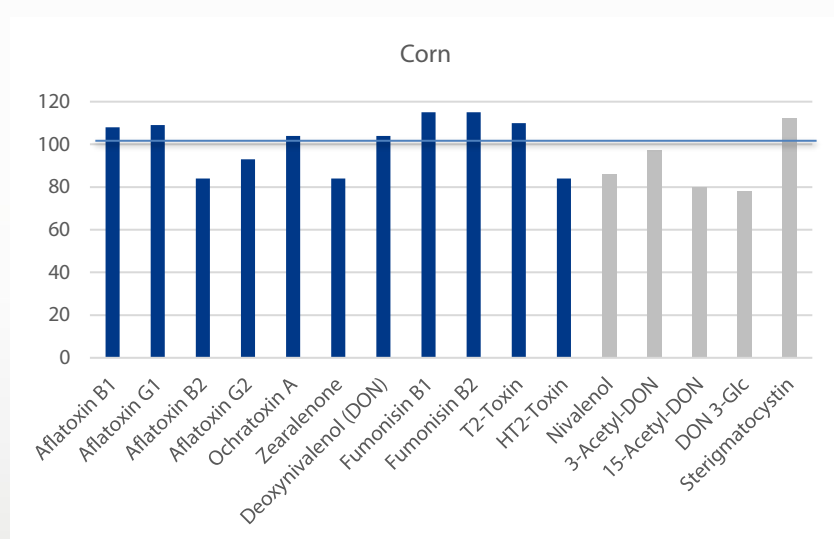


Figure 6: Recovery rates for Corn

3.2 Nuts or Nut-like Matrices

Table 3: Results for nuts / nut-like matrices (the abbreviations used in the table correspond in the order in which the diagrams are labelled)

	Afla B1	Afla G1	Afla B2	Afla G2	OTA	ZEA	DON	F-B1	F-B2	T2-Toxin	HT2-Toxin	NIV	3-Ac-DON	15-Ac-DON	DON 3-Glc	STC
Peanut	106	106	103	98	108	103	107	108	104	65	75	83	108	100	71	102
Hazelnut	114	105	107	104	101	86	96	106	109	102	71	110	108	89	88	110
Walnut	108	109	108	82	103	100	111	101	105	87	85	83	87	76	82	102
Pistachio	103	108	109	96	100	107	107	102	96	107	103	81	106	101	81	106
Almonds	105	101	106	106	109	100	91	104	103	78	82	104	108	92	79	102

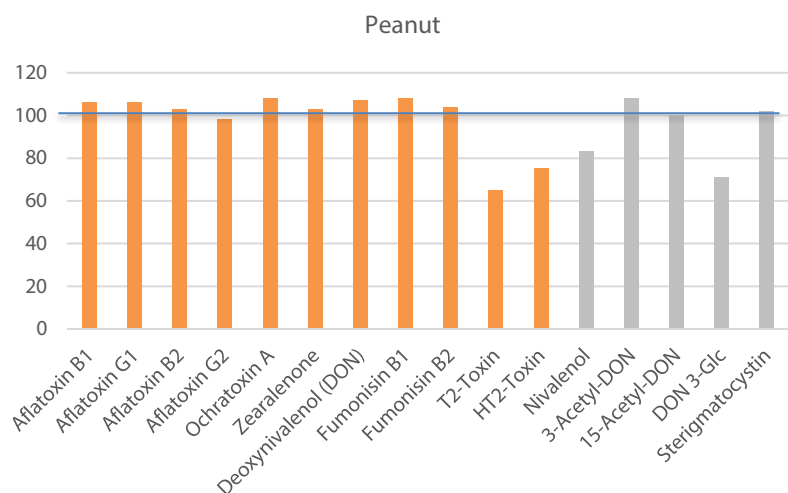


Figure 7: Recovery rates for Peanut

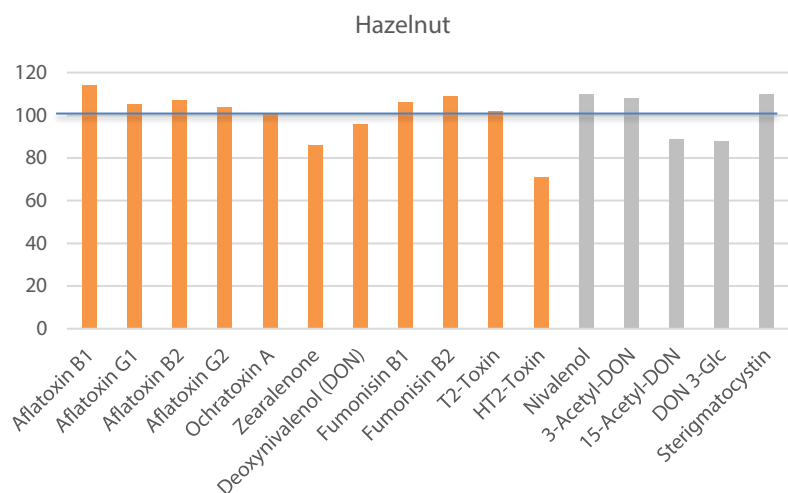


Figure 8: Recovery rates for Hazelnut

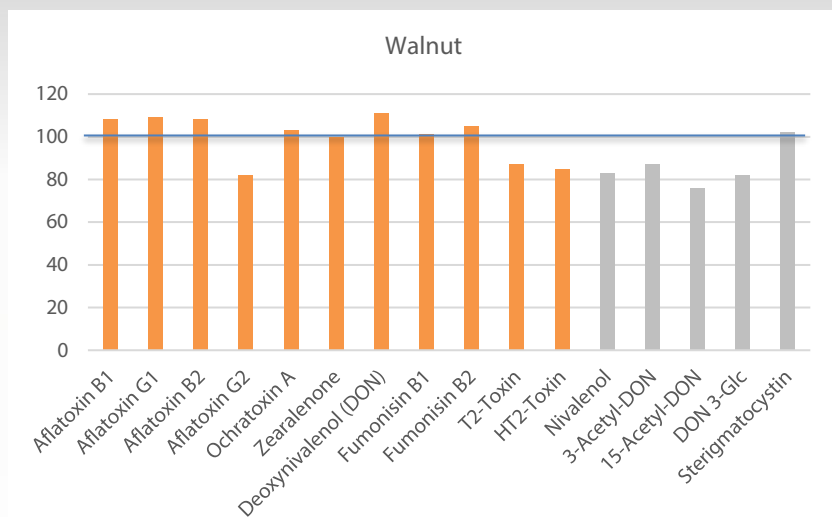


Figure 9: Recovery rates for Walnut

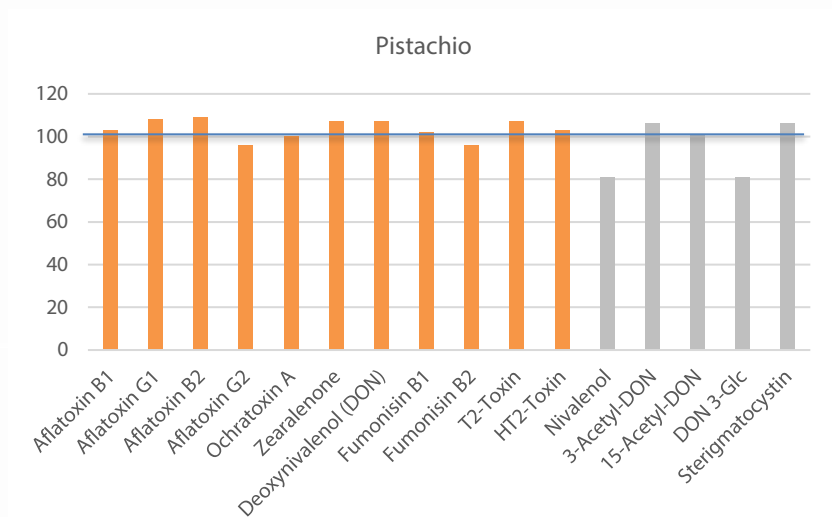


Figure 10: Recovery rates for Pistachio

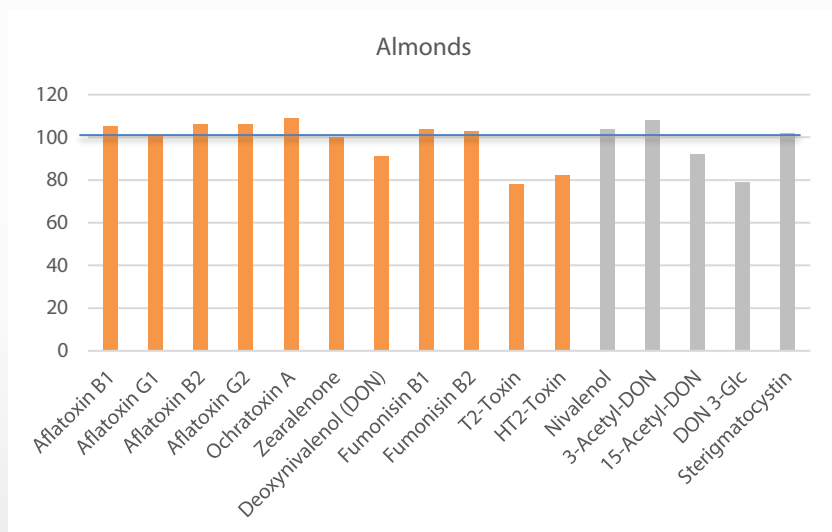


Figure 11: Recovery rates for Almonds

3.3 Dried Fruit Matrices

Table 4: Results for dried fruit matrices (the abbreviations used in the table correspond in the order in which the diagrams are labelled)

	Afla B1	Afla G1	Afla B2	Afla G2	OTA	ZEA	DON	F-B1	F-B2	T2-Toxin	HT2-Toxin	NIV	3-Ac-DON	15-Ac-DON	DON 3-Glc	STC
Raisins	106	108	109	108	103	84	93	101	102	84	89	89	105	98	89	102

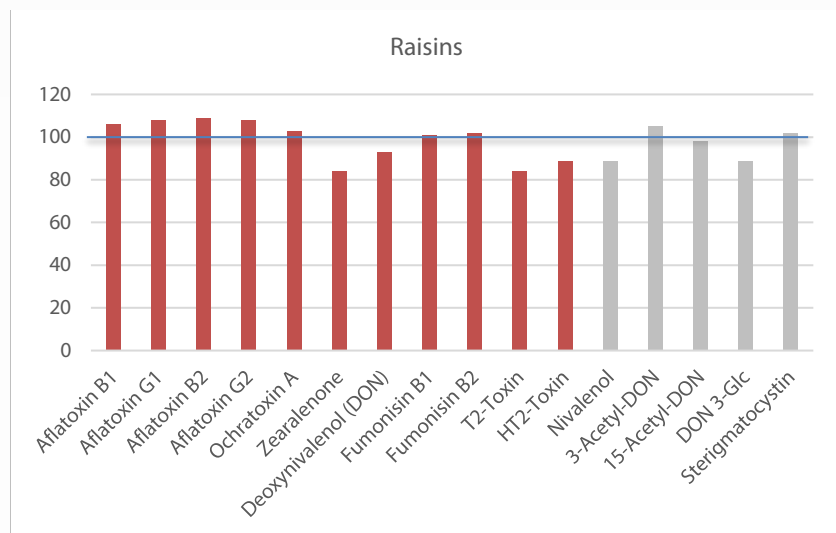


Figure 12: Recovery rates for Raisins

3.4 Spice Matrices

Table 5: Results for spice matrices

	Aflatoxin B1	Aflatoxin G1	Aflatoxin B2	Aflatoxin G2	Ochratoxin A
Bell Pepper / Chili	106	96	105	100	93
Ginger	74	93	106	146	76
Nutmeg	117	118	98	101	76
Turmeric	83	73	83	92	88

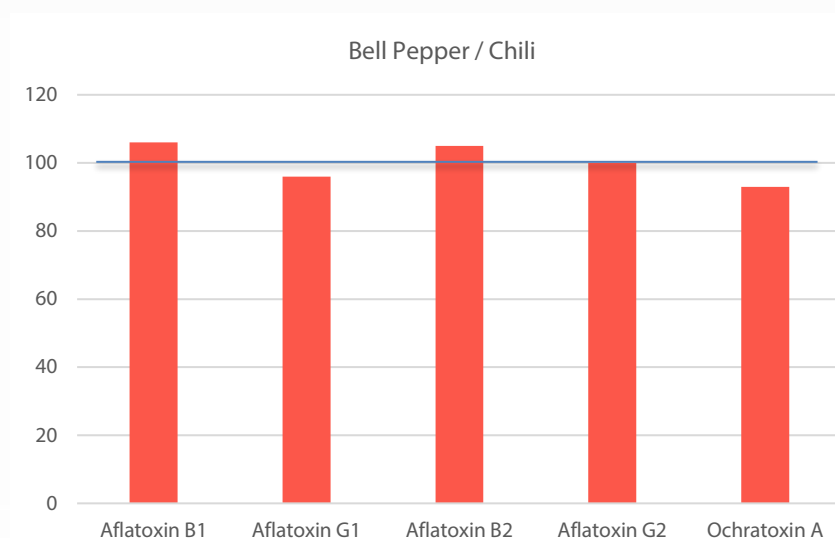


Figure 13: Recovery rates for Bell Pepper / Chili

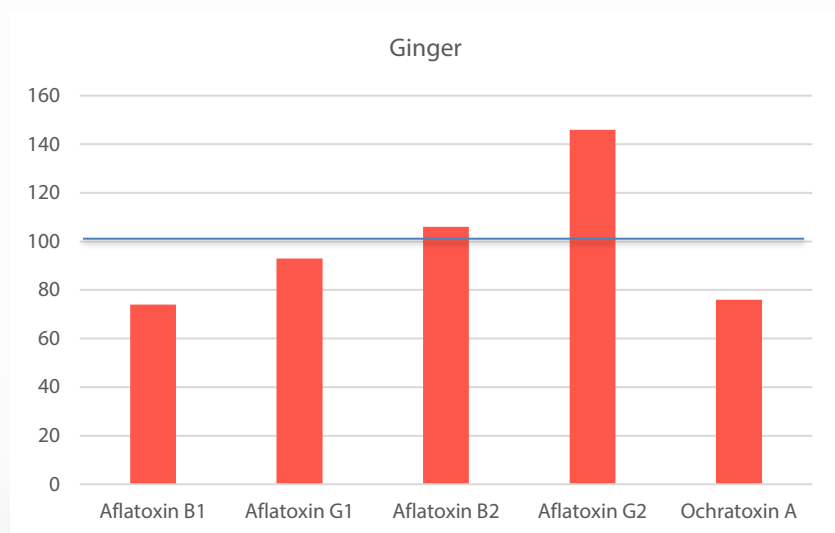


Figure 14: Recovery rates for Ginger

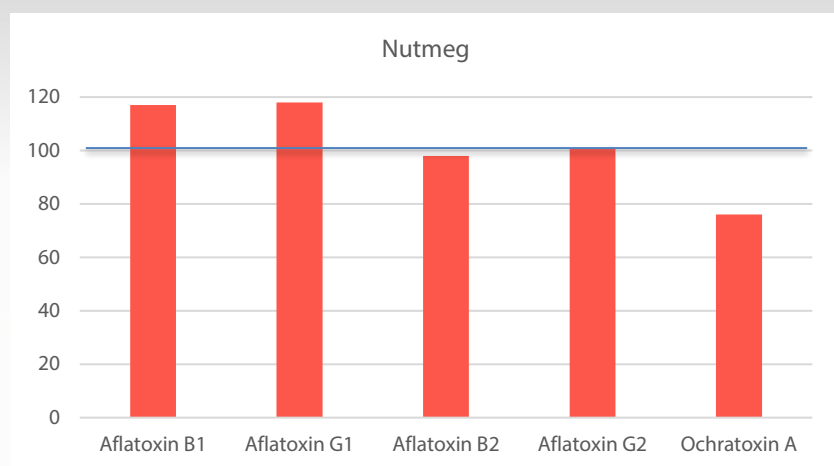


Figure 15: Recovery rates for Nutmeg

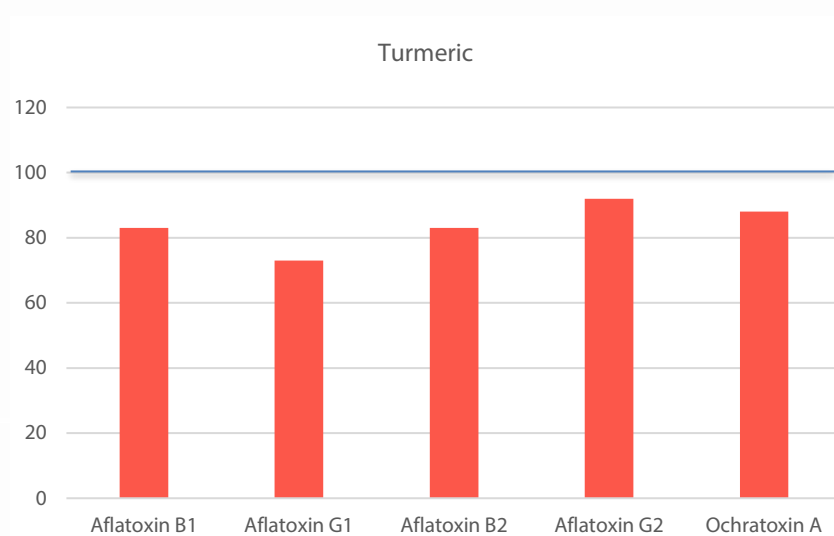


Figure 16: Recovery rates for Turmeric

3.5 Matrix Removal from Spice Samples – Reduction of Matrix Interferences

The matrix removal was observed for various matrices, matrix interferences and dye could be trapped in CrossTOX® column.



Figure 17: Chili (highly coloured matrix) - left not cleaned crude extract, right purified with CrossTOX® column



Figure 18: Chili (highly coloured matrix) - left unused CrossTOX® column, right used CrossTOX® column to clean-up the extract



Figure 19: Pistachio (low coloured matrix) - left not cleaned crude extract, right purified with CrossTOX® column



Figure 20: Pistachio (low coloured matrix) - left unused CrossTOX® column, right used CrossTOX® column to clean-up the extract

Matrix removal may lead to slight destaining of the sample, but the removal of matrix interferences, which inhibit LC-MS/MS analysis, is more dominant. Furthermore, the removal of matrix interferences lead to prolonged lifetime of the separation column and to higher analytical robustness of the LC-MS/MS and saving internal standards for analysis. The analytes are not retained in the CrossTOX® column, this allows efficient clean-up even with small sample volume.

The analytical interference of dyes in the LC-MS/MS is no indicator for effects on ionisation, because retention time and impact on product ion formation are not correlated with colour in general.

APPLICATION NOTE | AN0042

After 150 samples of spices and peanut, the sweep cone was checked and shows almost no matrix remains. This documents in a visible way how the clean-up with CrossTOX® improves the removal of matrix interferences, which in common application physically reduces system consistency.

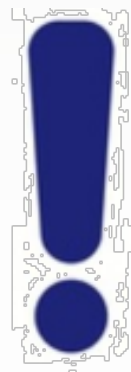


Figure 21: Sweep cone after 150 spice and peanut samples, which were cleaned-up by CrossTOX®.

The standards from the first (prior to matrix analysis) and after matrix analysis (150 samples) showed that in comparison with common application there is no drift, no changes in signal sensitivity, no interferences and no pressure increase in the LC, indicating the purity of the injected samples, and the compatibility of the clean-up with high throughput LC-MS/MS analysis.

4. Conclusion

The good recovery allows mycotoxin analysis not only from cereal but also from matrices containing higher fat or sugar content. The high matrix reduction and the robust sample clean-up allows standard reduction and increase in sample throughput for different food and feed mycotoxin analysis. By using the CrossTOX® column for sample preparation the sample number between maintenance intervals of your LC-MS/MS can be prolonged.



Figure 22: CrossTOX® columns

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