Effective Clean-up of 7 Regulated Mycotoxins and Determination by HPLC-MS/MS in grain

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Introduction

Mycotoxins are secondary metabolites of molds that can cause diseases in humans or animals. Har-vest losses due to mycotoxin infesta-tion can lead to negative economic impacts. The worldwide contamination of foods and feeds with mycotoxins is a significant problem [1]. The analysis of myco-toxins is challenging due to different problems like the wide range of chemical and physicochemical properties of the different types of mycotoxins. Additionally, numerous complicated matrices exist.

Fig. 1: Grain

They all contain molecules which can interfere with the analysis of mycotoxins. Sample preparation approa-ches are usually necessary [2]. The QuEChERS-me-thodology is a well-known technique for extraction of contaminants like pesticide residues in food and feed [3]. A fast, easy and cost-effective approach for the clean-up of sev-en regulated mycotoxins in different matrices is demonstrated. Effective cle-an-up of strongly matrix-contaminated samples can be achieved by adapting the mix composition to sample matrix. This method allows to detect and accurately quantify mycotoxin contents at low levels in e.g. wheat or rye flour. Also, the influence of syrin-ge filters on recovery rates are demonstrated.

Dispersive solid phase extraction (dSPE)

Extraction

- Weigh 4 g homogenized sample into a 50 mL centrifuge tube
- Add 25 µL mycotoxin standard mixture (β = 0.1 µg/mL each analyte in acetonitrile)
- Add 10 mL 0.1 % formic acid in water, shake vigorously and wait 10 min
- Add 10 mL acetonitrile and agitate
- Add CHROMABOND® QuEChERS Mix XII, shake vigorously for 1 min and cool the mixture down in an ice bath
- Centrifuge at 4500 rpm for 20 min at 20 °C
- Take organic phase for clean-up procedure

Clean-up

- Add 6 mL of organic phase into centrifuge tube with CHROMABOND® QuEChERS Mix M1 or Mix M2
- Shake vigorously for 1 min
- Centrifuge at 4500 rpm for 20 min at 20 °C
- Evaporate 2 mL extract to dryness at 60 °C under a stream of nitrogen and redissolve in 0.5 mL acetonitrile

Subsequent analysis HPLC-MS / MS

Chromatographic Conditions

Column 1:	EC 100/3 **NUCLEOSHELL® Phenyl-Hexyl**, 2.7 µm, (REF 763734.30)
Column 2:	EC 50/3 **NUCLEOSHELL® Bluebird RP 18**, 2.7 µm, (REF 763432.30)
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in acetonitrile
Gradient:	hold 5 % B for 1 min, from 5 % B to 40 % B in 3.0 min, from 40 % B to 95 % B in 1.0 min, hold 95 % B for 1 min, from 95 % B to 5 % in 1.0 min, hold 5 % B for 3.0 min
Flow rate:	0.56 mL/min
Temperature:	40 °C
Injection volume:	3 µL
MS conditions:	API 5500, ion source ESI, positive ionization mode, scan type MRM Curtain gas 20 psig, ion spray voltage 5500 V, temperature 550 °C, nebulizer gas 45 psig, turbo gas 45 psig, CAD medium

MRM Transitions

Analyt	$[M+H]^+$	Q1 (Quantifier)	Q2 (Qualifier)
Aflatoxin B1	313.0	285.0	241.0
Aflatoxin B2	315.1	287.0	258.9
Aflatoxin G1	329.0	242.9	311.0
Aflatoxin G2	331.1	313.0	244.9
HT-2 toxin	425.2	263.0	215.0
T-2 toxin	467.2	305.0	244.9
Zearalenone	319.1	187.1	185.0

Table 1: MRM transitions for mycotoxins

Syringe Filter Test

Analyt	H-PTFE 0.45 µm	PA 0.20 µm	PES 0.20 µm	PET 0.20 µm	PTFE 0.20 µm	PVDF 0.20 µm	RC 0.20 µm
Aflatoxin B1	105 ± 3 %	79 ± 9 %	90 ± 2 %	100 ± 3 %	98 ± 3 %	29 ± 9 %	109 ± 7 %
Aflatoxin B2	101 ± 5 %	72 ± 5 %	88 ± 2 %	96 ± 6 %	94 ± 5 %	33 ± 11 %	95 ± 3 %
Aflatoxin G1	102 ± 5 %	73 ± 11 %	86 ± 5 %	93 ± 3 %	91 ± 1 %	33 ± 10 %	99 ± 6 %
Aflatoxin G2	105 ± 2 %	81 ± 3 %	98 ± 1 %	98 ± 2 %	96 ± 4 %	39 ± 6 %	102 ± 3 %
HT-2 toxin	102 ± 2 %	106 ± 5 %	106 ± 2 %	99± 10 %	102 ± 1 %	92 ± 6 %	100 ± 5 %
T-2 toxin	97 ± 2 %	90± 3 %	90 ± 1 %	97 ± 3 %	95± 5 %	17 ± 37 %	95 ± 0 %
Zearalenone	100 ± 3 %	95 ± 2 %	89 ± 0 %	97 ± 2 %	90 ± 2 %	5 ± 34 %	94 ± 1 %
Fumonisin B1	5 ± 9 %	1 ± 20 %	2 ± 12 %	57 ± 4 %	3 ± 27 %	13 ± 3 %	52 ± 5 %
Fumonisin B2	6 ± 6 %	1 ± 22 %	2 ± 10 %	48 ± 8 %	3 ± 29 %	10 ± 7 %	41 ± 12 %

Table 2: Recovery rates using different types of Syringe Filters

Recovery rates

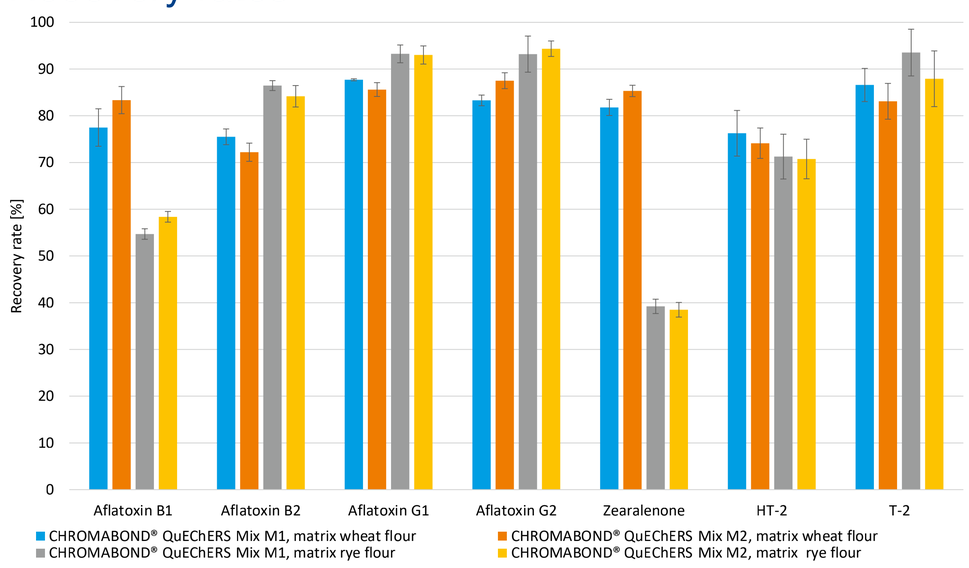

Fig. 2: Recovery rates of mycotoxins measured on NUCLEOSHELL® Bluebird RP 18 column.

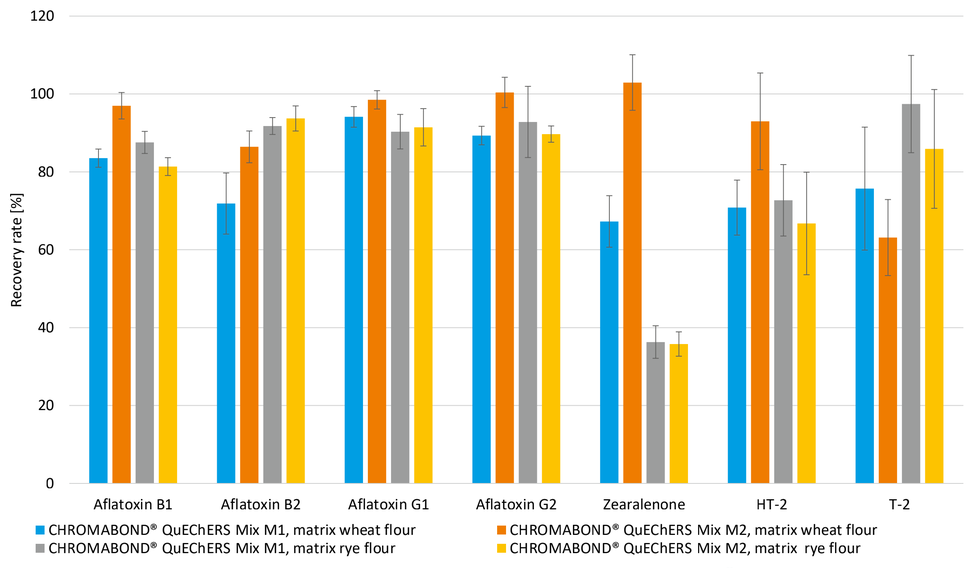

Fig. 3: Recovery rates of mycotoxins measured on NUCLEOSHELL® Phenyl-Hexyl column.

Matrix reduction

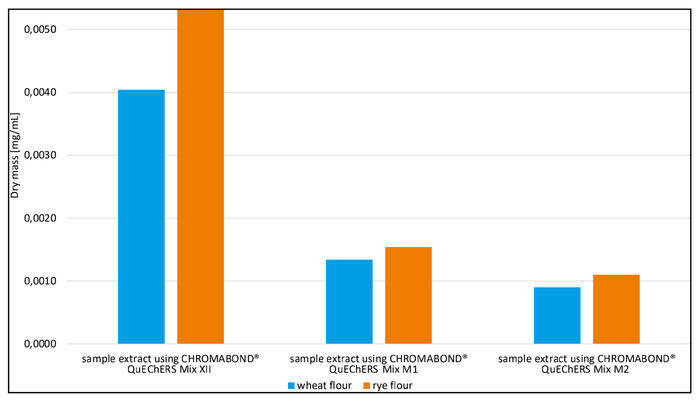

Fig. 4: Reduction of dry mass for sample matrices wheat flour und rye flour.

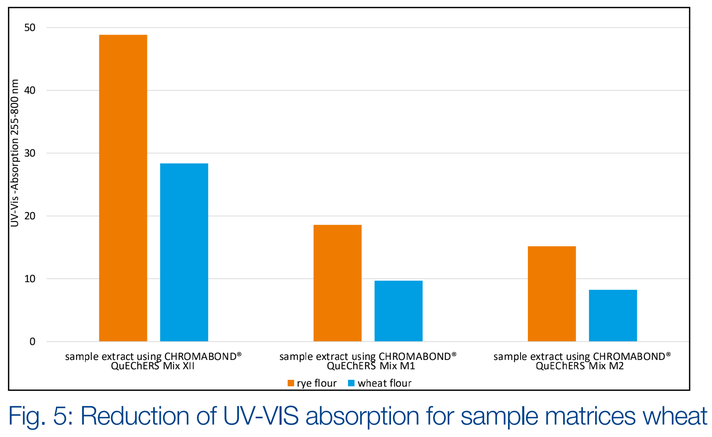

Fig. 5: Reduction of UV-VIS absorption for sample matrices wheat flour und rye flour.

Conclusion

Successful clean-up of seven regulated mycotoxins can be conducted with both QuEChERS cle-an-up Mixes M1 and M2. By using CHROMABOND® QuEChERS clean-up Mix M2 the highest matrix reduction could be achieved for both sample matrices. The reduction of dry mass and of UV-VIS-Absorption is significant for both tested products. Whereas the recovery rates for ZEA are significantly better in wheat flour than in rye flour. The better reproducibility and the homogeneous recovery rates are achieved on NUCLEOSHELL® Bluebird RP 18 due to the better chromatographic performance. The syringe filter test has shown that the type of membrane has to be chosen care-fully. Especially PVDF membrane should be avoided for mycotoxin analysis.

References

[1] M.E. Zain, Journal of Saudi Chemical Society, Volume 15, Issue 2, April 2011, 129-144
[2] L. Zhang, X.W. Dou, C. Zhang , A.F. Logrieco, and M-H Yang, Toxins 2018, 10, 65
[3] M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, J. AOAC Int. 86 (2003), 412–431.

Product information

The following MACHEREY-NAGEL products have been used in this application:

REF 763734.30, EC 100/3 NUCLEOSHELL® Phenyl-Hexyl, 2.7µm
REF 763432.30, EC 50/3 NUCLEOSHELL® Bluebird RP 18, 2.7µm
REF 730648, CHROMABOND® QuEChERS Mix XII
CHROMABOND® QuEChERS Mix M1, order on request
CHROMABOND® QuEChERS Mix M1, order on request
Various CHROMAFIL® Syringe Filters