New Developments in the Analysis of Mycotoxins in Cereal-Based Food

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Introduction

Cereal-based products constitute the staple diet in many countries. Unfortunately these products are often contaminated with Fusarium toxins by infection of cereal crops with fungal plant pathogens mainly by *Fusarium graminearum* during the growth period. Depending on the storage and the climate conditions there is also the risk of contamination with Aflatoxins and Ochratoxin A. These toxins are produced by several Aspergillus and Penicillium species, especially *Aspergillus flavus*, *Aspergillus ochraceaus* and *Penicillium verrucosum* and they are serious health hazards to the consumers, especially to babies and infants.
The objective of our study was to develop multi component methods for the simultaneous quantification of Fusarium toxins (Trichothecenes A/B, Zearalenone, Fumonisins B1 and B2) as well as Aflatoxins and Ochratoxin A in cereal-based food by LC-MS/MS according to the requirements of the Commission Regulation (EC) 1881/2006 and the German Diet Regulation (DiätV).

Experimental

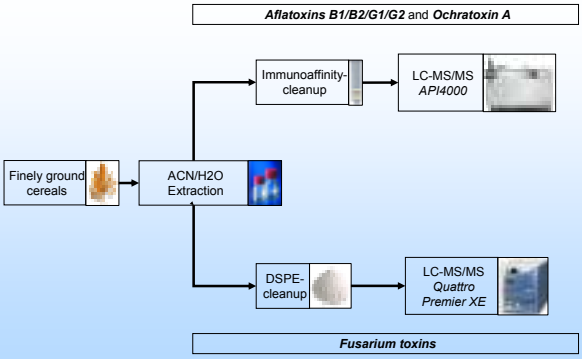

Fig .1 sample preparation scheme

The extraction of the ground cereal-based material was performed with a polar solvent mixture with a defined water content followed by dispersive solid phase extraction (DSPE) cleanup. The determination was accomplished by UPLC-MS/MS (Quattro Premier XE™, Waters).
To achieve very low LOQs for the Aflatoxins and Ochratoxin A immunoaffinity columns (AflaOchra™, Vicam) were used for cleanup and enrichment of sample extracts. For determination of these toxins the LC-MS/MS (API4000™, Applied Biosystems) was used in MRM mode.

Tab.1 LC instrumental data

1. LC-Series 1100 (Agilent)

Column:	Ascentis Express 3.0x100mm 2.7 µm
Flow:	0,4 mL/min
Gradient	ACN/H2O
Injection Volume	10 µL
Oven Temperature	60 °C
Runtime	15 min

2. Acquity UPLC-System (Waters)

Column:	ACQUITY UPLC BEH Phenyl 2.1x50mm 1.7µm
Flow:	0,4 mL/min
Gradient	MeOH/H2O
Injection Volume	3 µL
Oven Temperature	60 °C
Runtime	6.5 min

Tab.2 MS parameters API4000 (Applied Biosystems)

Toxin	m/z Parent	Daughter 1	Daughter 2	Polarity
Aflatoxin B1	313,3	285,0	241,1	+
Aflatoxin B2	315,2	287,1	259,1	+
Aflatoxin G1	329,2	243,0	214,3	+
Aflatoxin G2	331,0	313,0	285,0	+
Ochratoxin A	403,9	313,0	220,9	+

Tab.3 MS parameters Quattro Premier XE (Waters)

Toxin	m/z Parent	Daughter 1	Daughter 2	Polarity
Deoxynivalenol	297,0	249,2	203,1	+
3-Acetyldeoxynivalenol	339,0	231,1	104,9	+
15-Acetyldeoxynivalenol	356,0	321,1	136,9	+
Fusarenon X	372,0	247,2	229,1	+
Nivalenol	313,1	205.1	175,3	+
Diacetoxyscirpenol	383,0	307,1	199,0	+
HT-2 Toxin	447,1	345,1	285,1	+
T-2 Toxin	489,1	387,1	245,1	+
Zearalenone	317,3	130,9	174,9	-
Fumonisin B1	722,4	352,4	334,3	+
Fumonisin B2	706,4	336,4	318,4	+

Results

The validation data for the mycotoxins were determined in wheat according to DIN 32645.

For the Aflatoxins and Ochratoxin A the LOQs were in the range of 0,003 to 0,01 µg/kg. For this reason the method is suitable to determine trace concentrations of these critical compounds in one single run with factors 3 - 50 below the values that are defined as maximum levels of Aflatoxins and Ochratoxin A in cereal based infant food by the German Diet Regulation and the Commission Regulation (EC) 1881/2006.

The LOQs of the Fusarium toxins were in the range of 5 to 50 µg/kg. With this new method it is possible to detect all important Fusarium toxins in concentration levels factor 5 - 12 below the requirements of the German Diet Regulation and the Commission Regulation (EC) 1881/2006 by UPLC within a runtime of 6,5 min.

For the determination of Fusarium toxins matrix-matched calibration is recommended because of variably strong ion suppression effects in different matrices.

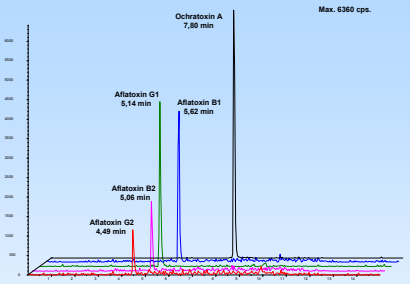

Fig.2 Spiked wheat sample (AFB1/G1 = 0,015 µg/kg, AFB2/G2 = 0,0045 µg/kg, OTA = 0,06 µg/kg) after cleanup with immunoaffinity column aquired by LC-MS/MS (API4000)

Tab.4 Validation data according to DIN 32645 aquired by API 4000 after IAC-cleanup

Toxin	RT [min]	LOD [µg/kg]	LOQ [µg/kg]	Recovery [%]	CV [%]	Maximum levels [µg/kg] DiätVO	(EC)1881/2006
Aflatoxin B1	5,62	0,001	0,003	92	1,1		0,1[2]
Aflatoxin B2	5,06	0,001	0,003	87	1,6	Sum Total Aflatoxins B/G 0,05[1]	---
Aflatoxin G1	5,14	0,001	0,003	90	1,3		---
Aflatoxin G2	4,49	0,002	0,005	76	0,7		---
Ochratoxin A	7,80	0,004	0,01	83	1,2	---	0,5[2]

[1] Dietary food for infants and young children [DiätVO]
[2] Processed cereal-based foods and baby foods for infants and young children [(EC) 1881/2006]
[3] Cereal products for production of dietary food for infants and young children [DiätVO]
[4] Maize products for production of dietary food for infants and young children [DiätVO]
[5] Processed maize-based foods and baby foods for infants and young children [(EC) 1126/2007]

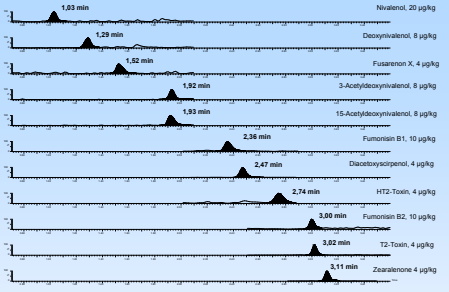

Fig.3 Spiked wheat sample after cleanup with DSPE, aquired by LC-MS/MS (Quattro Premier XE)

Tab.5 Validation data according to DIN 32645 aquired by Quattro Premier XE

Toxin	RT [min]	LOD [µg/kg]	LOQ [µg/kg]	Recovery [%]	CV [%]	Maximum levels [µg/kg] DiätVO	(EC)1881/2006
Deoxynivalenol	1,29	4,9	8,4	78	0,7	100 [3]	200 [2]
3-Acetyldeoxynivalenol	1,92	7,9	13,4	89	1,1	---	---
15-Acetyldeoxynivalenol	1,93	8,6	14,4	90	1,2	---	---
Fusarenon X	1,52	6,5	11,1	82	0,7	---	---
Nivalenol	1,03	12,9	21,2	74	0,8	---	---
Diacetoxyscirpenol	2,47	2,5	4,4	92	0,7	---	---
HT-2 Toxin	2,74	6,5	10,8	87	1,7	---	---
T-2 Toxin	3,02	4,9	8,3	95	1,3	---	---
Zearalenone	3,11	0,9	1,7	93	0,5	20 [3]	20 [2]
Fumonisin B1	2,36	5,6	9,4	78	1,5	Sum FB1/FB2 100 [4]	Sum FB1/FB2 200 [5]
Fumonisin B2	3,00	2,1	3,6	75	1,4		

Conclusion

- Newly developed and validated method for simultaneous quantification and confirmation of all important mycotoxins in cereal-based products
- Highest sensitivity, especially for determination of Aflatoxins and Ochratoxin A in one single run down to ppt levels
- Fusarium toxin multi component method with very short analysis time due to the high performance of the UPLC-System