Mycotoxins – analyzing an interesting and challenging group

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Introduction:

Mycotoxins are a class of compounds that are produced by mold. Due to their high toxicity to vertebrates it is important to monitor the concentrations of mycotoxins in food and feed. Over a hundred mycotoxins are known with Aflatoxins (B1, B2, G1, G2), Zearalenon, DON, T2-Toxin, HT2-Toxin, Ochratoxin A and Fumonisins being the ones of the highest concern.

Here we report a fast, easy and reliable method with the option of screening an unlimited number of mycotoxins in general. We report results for the most important mycotoxins showing that QTOF MS is a very sensitive technique to cover a broad range of compound classes of interest for routine analysis.

Methods:

- Maize samples from a proficiency test were prepared after a QuEChERS method.
- Multistep gradient, runtime 20 min, A (water/ MeOH, 90:10) with 5 mM NH_4HCO_2 and 0.01% formic acid, B (MeOH) with 5 mM NH_4HCO_2 and 0.01% formic acid.
- Analysis were performed using an UltiMate 3000 (Thermo, Dionex) LC system coupled to a compact QTOF mass spectrometer (Bruker Daltonics).
- MS Data acquired from 50-1000 m/z in alternating full-scan and broad-band CID acquisition mode at 2 Hz in positive ionization mode.
- Calibration curves were prepared in solvent A (with ^{13}C labeled internal standards). Samples were spiked with ^{13}C labeled internal standards to compensate for matrix effects.
- Processing was done in TASQ 1.4 (Bruker Daltonics).

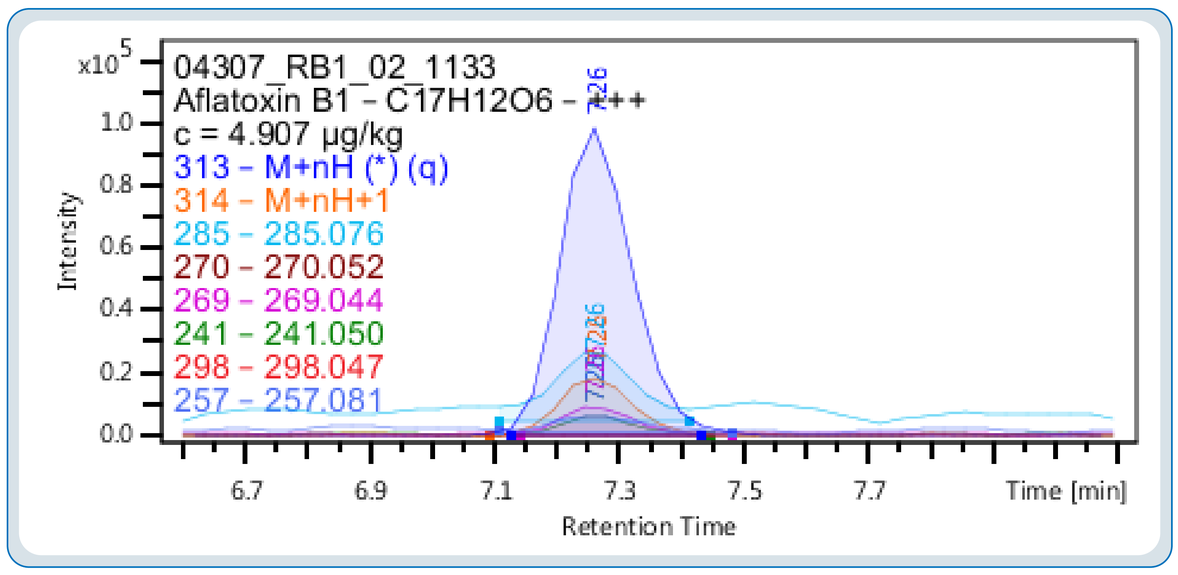

Fig. 2 Chromatogram traces for Aflatoxin B1 of a representative proficiency test sample. It can be clearly seen that besides the principal ion ($[M+H]^+$ in dark blue) the different qualifier ions (all other colors) can be clearly seen. A concentration of 4.91 µg/kg suits perfectly to the spiked value of 4.81 µg/kg.

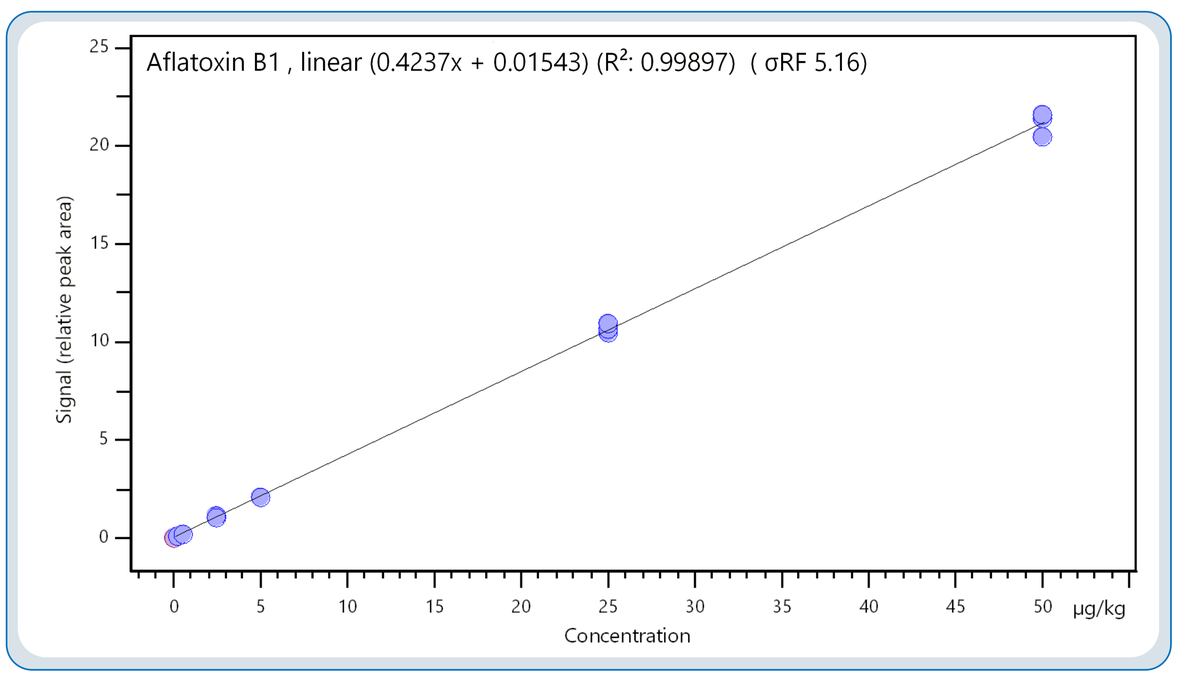

Fig. 1 Calibration curve for Aflatoxin B1 of triplicate measurements from 0.25-50 µg/kg in solvent A.

Mykotoxin	LOD	LOQ	R2	Linear range
	µg/kg	µg/kg		µg/kg
Aflatoxin B1	<0.25	0.25	0.99897	0.25-5
Aflatoxin B2	<0.25	0.25	0.99961	0.25-50
Aflatoxin G1	<0.25	0.25	0.99885	0.25-50
Aflatoxin G2	<0.25	0.25	0.99992	0.25-25
DON	0.25	0.50	0.99960	0.5-1000
HT2-Toxin	<0.25	2.50	0.99978	2.5-250
Ochratoxin A	0.5	0.50	0.99876	0.5-2500
T2-Toxin	1	2.00	0.99973	2-2000
Zearalenone	<10	10.00	0.99976	10-2000

Table 1: Result summary of the detected and quantified mycotoxins using TASQ 1.4 (Bruker Daltonics). All calibration curves show a linear fit within a 20% precision.

Results:

- The herein described methods are based on the complete solution PesticideScreener 2.1 (Bruker Daltonics). Even though the method is optimized for pesticides it could be perfectly transferred to mycotoxins applying only slight changes (e.g. collision energy). The achieved linear ranges, LOQs and LODs were shown to be suitable for the regulated concentrations.
- Calibration curves were measured in solvent A. To avoid any matrix effects, a fully ^{13}C labeled internal standard for each mycotoxin was spiked.
- All mycotoxins could be quantified using a linear calibration curve fit with R^2 values > 0.9987.
- Fig. 1 shows a calibration curve for Aflatoxin B1. A linear fit for 0.25-50 µg/kg shows a high R^2 value.
- Fig. 2 shows the chromatogram traces in the proficiency maize test sample for Aflatoxin B1. It can be clearly seen, that the principal ion ($[M+H]^+$) was nicely detected and verified by a number of different fragments (qualifier ions).
- Aflatoxin B1 was spiked with 4.81 µg/kg in the sample. Quantitation of this sample resulted in a concentration of 4.91 µg/kg which is in good alignment to the expected value (accuracy: 102%).
- All other mycotoxins were tested and quantified with good accuracies during the proficiency test: Aflatoxin G2 (0.3 µg/kg), DON (1195.2 µg/kg, 104%), Ochratoxin (2.2 µg/kg, 72%) and ZEN (194.8 µg/kg, 105%).

http://www.istockphoto.com/de/foto/corn-rot-gm185962455-27416865

Conclusions:

- All mycotoxins can be identified and quantified down to concentrations that are regulated by authorities.
- Some mycotoxins were identified and quantified down to 0.25 µg/kg with high accuracies.
- Fully ^{13}C labeled internal standards compensate for matrix effects. Therefore, no matrix matched calibration is needed which simplifies the routine work, tremendously

hr-QTOF-MS