Determination of mycotoxins in food and feed with UHPLC-QqQ-MS/MS and UHPLC-QTOF-MS

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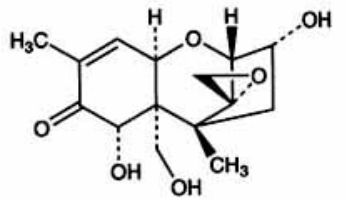
Mycotoxins



- mycotoxins are low molecular weight, toxic, secondary metabolites of fungi
- *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria* spp.
- >25% of all agricultural goods are contaminated
- annual losses of several hundred million tons of food worldwide
- annual economical losses: 1 billion USD (US only)
- 100+ countries have regulations for the control of mycotoxins in food and feed

Reasons

Why do we need multi-mycotoxin methods?

- unified methods saves time and costs

- for regulated mycotoxins
  - high degree of accuracy
  - easy to handle, cost effective
  - limits of quantitation below the maximum limits

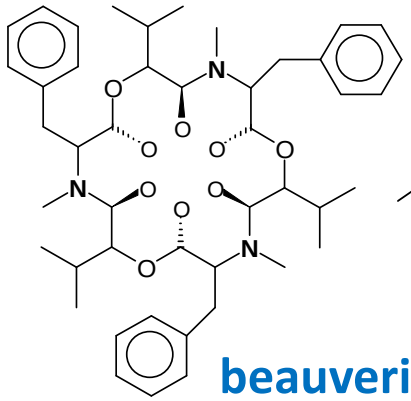
Stable Isotope Dilution Assay (SIDA)

multi-toxin method for around 200 metabolites

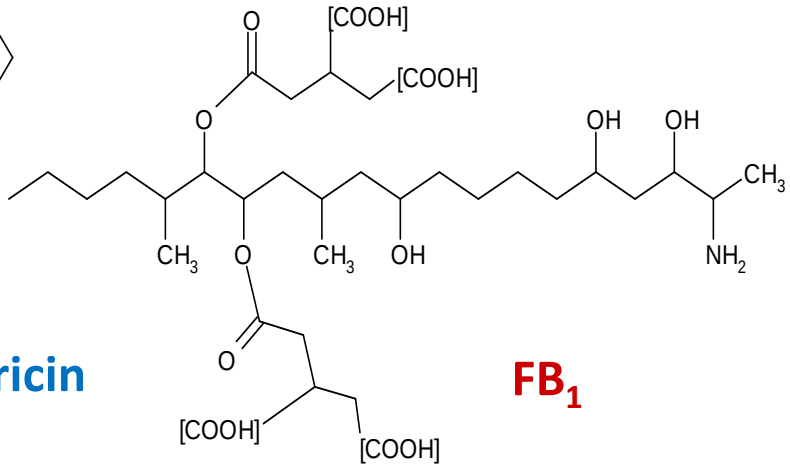
- for a huge variety of mycotoxins
  - identification of mycotoxins in unlikely matrices
  - analysis of food and feed mixtures of several commodities

Challenges

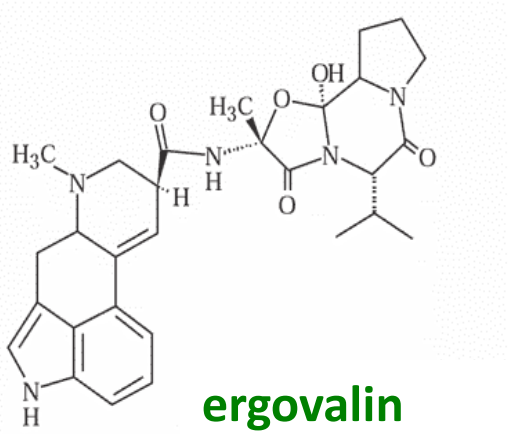
- chemical diversity of the investigated analytes



beauvericin



FB_1

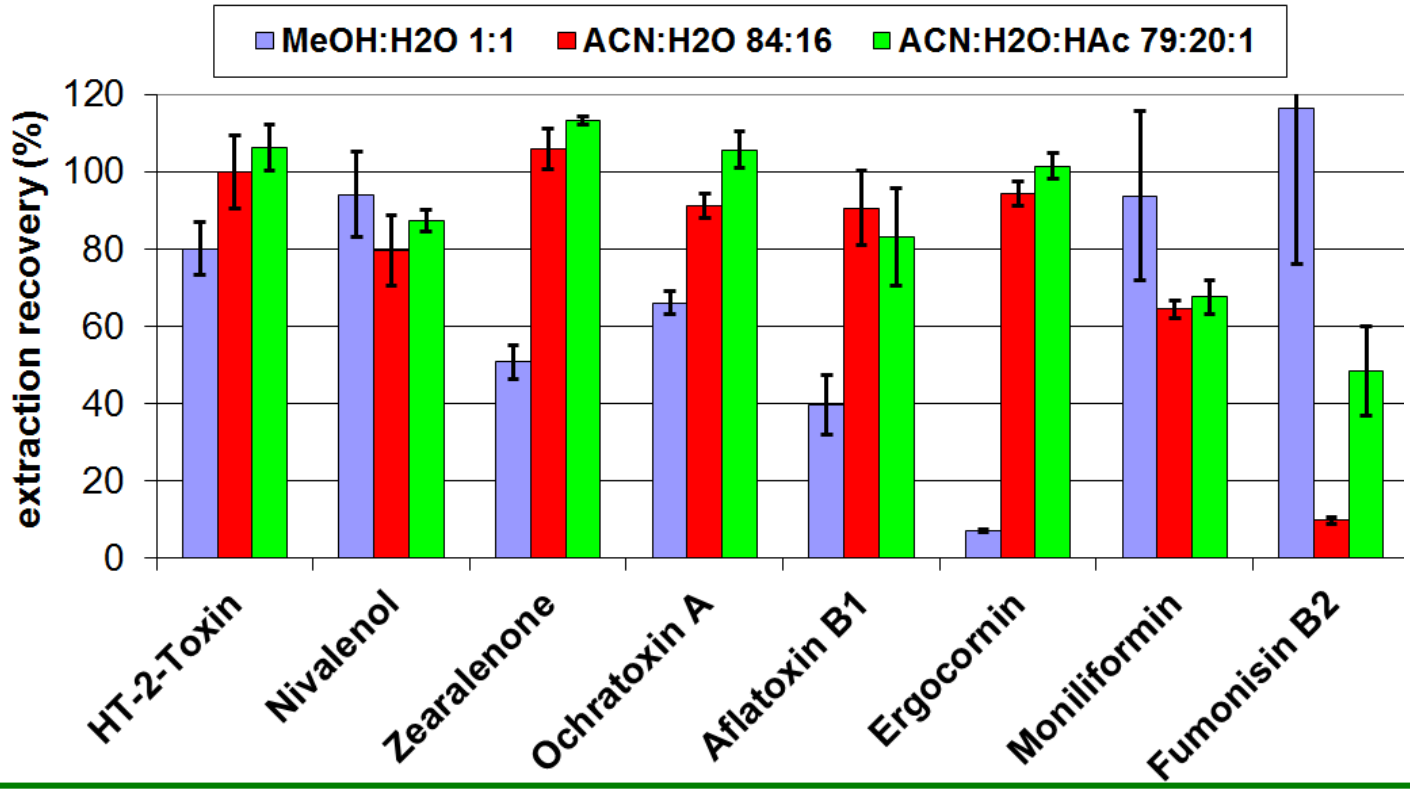


ergovalin

Enniatins	Fumonisins	Ergot alkaloids
beauvericin enniatin A, A_1, B, B_1	fumonisin B_1, FB_2, FB_3, hydrolyzed FB_1	ergotamin, ergocornin, ergovalin, dihydroergosin
APOLAR	POLAR, ACIDIC	POLAR, BASIC

Solutions

- general sample preparation
  - careful optimisation of extraction solvents



e.g. wheat

n=3

sample: solvent ratio (1:4)

extract diluted 1:1

best compromise: low water content, low pH ⇒ ACN:H_2O:HAc (79:20:1, v:v:v)

Sulyok M, Berthiller F, Krska R, Schuhmacher R. (2006), Rapid Commun. Mass Spectrom. 20(18):2649-2659

Solutions

- general sample preparation
  - careful optimisation of extraction solvents
  - common sample clean-up difficult
    always potential to loose analytes
    → injection of (diluted) crude extracts

- general separation and detection principle
  - LC-MS/MS
  - wide range of relevant concentrations:
    e.g. regulated mycotoxin in the EU:
    2 µg/kg (AFB_1) - 1750 µg/kg (DON)
  - use of high end analytical equipment
    (sensitivity, linearity)

Accurate Stable Isotope Dilution Assay (SIDA) for mycotoxins regulated in the European Union

Approaches

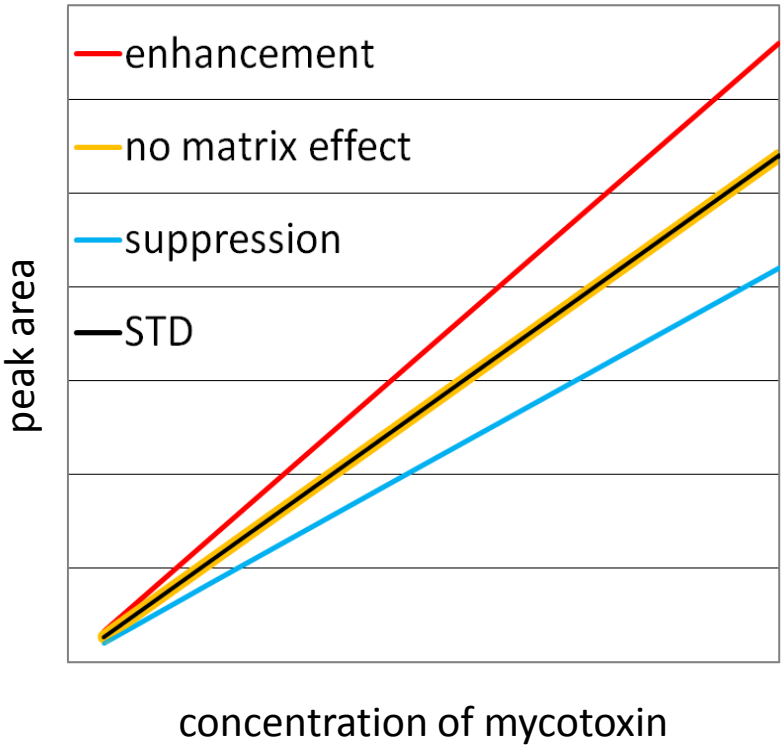
- European Commission Regulation (EC) No 1881/2006

BUT:

- electrospray ionisation (ESI)
  - matrix effects may hamper accurate mass spectrometric quantification

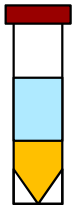
Approaches:

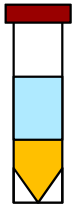
- dilution of the sample
- matrix matched calibration
- standard addition to each sample
- internal calibration
  - ideally: behaves exactly the same as analyte, but still distinctive
  - [^{2}H]- or [^{13}C]-labelled compounds

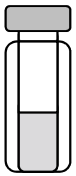


Sample Preparation

Milling	grind and homogenise sample + weight-in
1. Extraction	acetonitrile:water:formic acid (80:19.9:0.1, v:v:v) 60 min at room temperature on a rotary shaker
Centrifugation	
2. Extraction	acetonitrile:water:formic acid (20:79.9:0.1, v:v:v) 30 min at room temperature on a rotary shaker
Centrifugation	
+ISTD Transfer	transfer 80 µL aliquot to HPLC vial with microinsert and add 20 µL ISTD-mix
Analysis	UHPLC-MS/MS

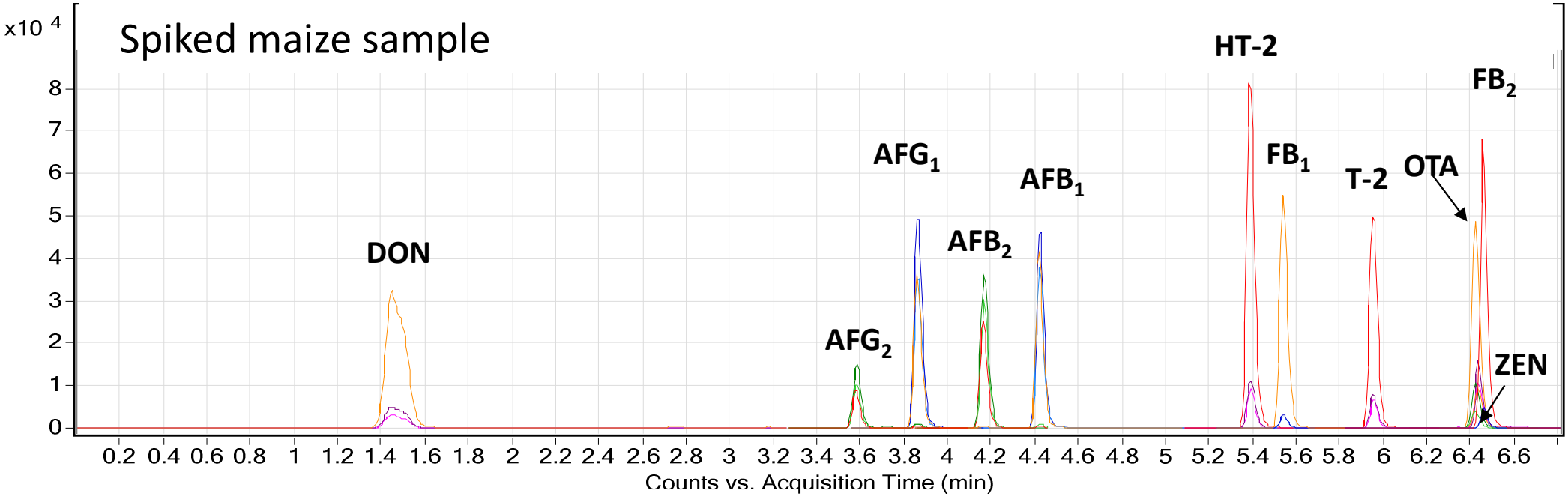






UHPLC-MS/MS Setup

- 1290 Infinity UHPLC (Agilent)
  - column: Zorbax RRHD Eclipse Plus C18
  - A: H_2O / 0.1% HCOOH / 5mM NH_4COOH
  - B: MeOH / 0.1% HCOOH / 5mM NH_4COOH
- 6490 QqQ MS/MS (Agilent)
  - single run, fast polarity switching, dynamic MRM



Results – Validation

Analyte	External calibration R_A [%] ± RSD [%] [1)]
Aflatoxin B_1	35 ± 4
Aflatoxin B_2	42 ± 4
Aflatoxin G_1	46 ± 5
Aflatoxin G_2	40 ± 6
Deoxynivalenol	41 ± 10
Fumonisin B_1	330 ± 6
Fumonisin B_2	181 ± 7
HT-2 toxin	134 ± 6
Ochratoxin A	167 ± 14
T-2 toxin	118 ± 5
Zearalenone	91 ± 6

[1)] Apparent recovery ± Relative Standard Deviation

suppression

enhancement

Results – Validation

Analyte	External calibration R_A [%] ± RSD [%] [1)]	Internal calibration R_A [%] ± RSD [%] [1)]	R_E [%] [2)]
Aflatoxin B_1	35 ± 5	105 ± 6	108
Aflatoxin B_2	45 ± 5	100 ± 4	107
Aflatoxin G_1	50 ± 4	101 ± 5	109
Aflatoxin G_2	43 ± 8	101 ± 8	111
Deoxynivalenol	49 ± 6	96 ± 5	106
Fumonisin B_1	356 ± 10	101 ± 10	103
Fumonisin B_2	180 ± 8	88 ± 7	97
HT-2 toxin	148 ± 7	98 ± 7	109
Ochratoxin A	168 ± 11	93 ± 7	102
T-2 toxin	127 ± 5	99 ± 6	110
Zearalenone	89 ± 10	103 ± 11	109

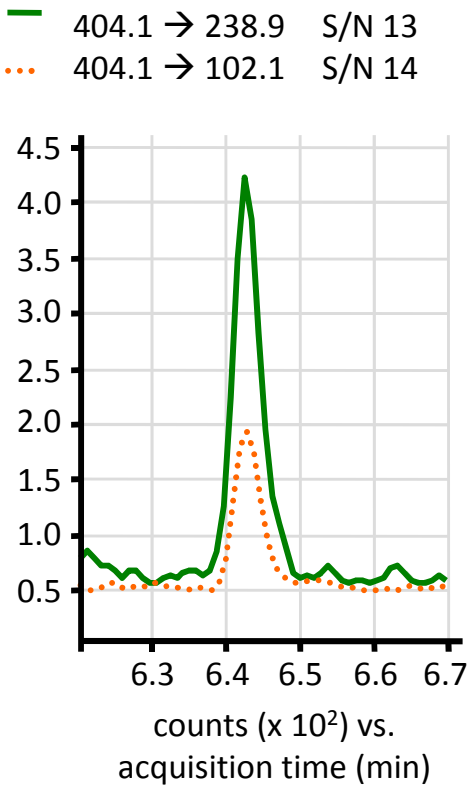
1) Apparent recovery ± Relative Standard Deviation

2) Extraction recovery

Results – LOQ

Analyte	LOQ (µg/kg) [1)]	MLs (µg/kg) [2)]
Aflatoxin B_1	0.1	2.0 – 5.0 (**0.1**)
Aflatoxin B_2	0.1	Sum AFs 4.0 – 10.0
Aflatoxin G_1	0.1	
Aflatoxin G_2	0.4	
Deoxynivalenol	11	500 – 1750 (200)
Fumonisin B_1	4.3	Sum FBs 800 – 4000 (200)
Fumonisin B_2	3.9	
HT-2 toxin	2.5	to be decided
Ochratoxin A	0.4	3.0 – 5.0 (**0.5**)
T-2 toxin	0.2	to be decided
Zearalenone	2.9	50 (20)

OTA: 0.45 µg/kg



[1)] Limit of Quantification (S/N = 10) for maize

[2)] Maximum Levels for various commodities according to European Union Commission Regulation 1881/2006 and its amendments (numbers in brackets: baby food)

Summary

- accurate determination of regulated mycotoxins in maize
- two-step extraction → enhanced the extraction recovery
- internal standards: compensate matrix effects, minimise costs
- sensitivity suitable for MLs

Anal Bioanal Chem (2012) 402:2675–2686
DOI 10.1007/s00216-012-5757-5

PAPER IN FOREFRONT

Stable isotope dilution assay for the accurate determination of mycotoxins in maize by UHPLC-MS/MS

Elisabeth Varga · Thomas Glauner · Robert Köppen · Katharina Mayer · Michael Sulyok · Rainer Schuhmacher · Rudolf Krska · Franz Berthiller

Open access

Agilent Technologies

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IFA TULLN

UHPLC-QqQ-MS/MS and UHPLC-QTOF-MS/MS methods for the (semi-)quantitative determination of mycotoxins

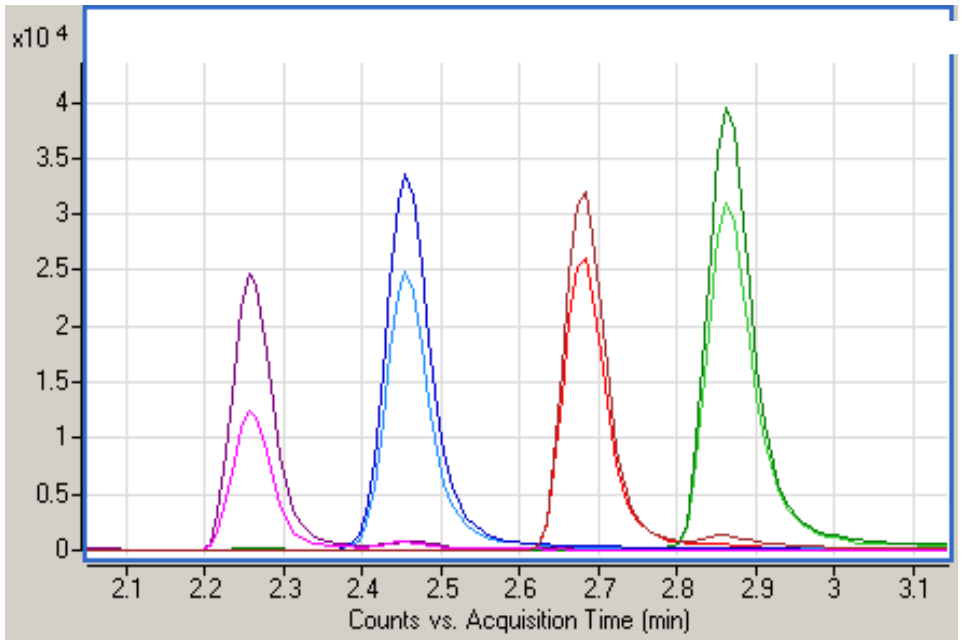
Multi-toxin methods

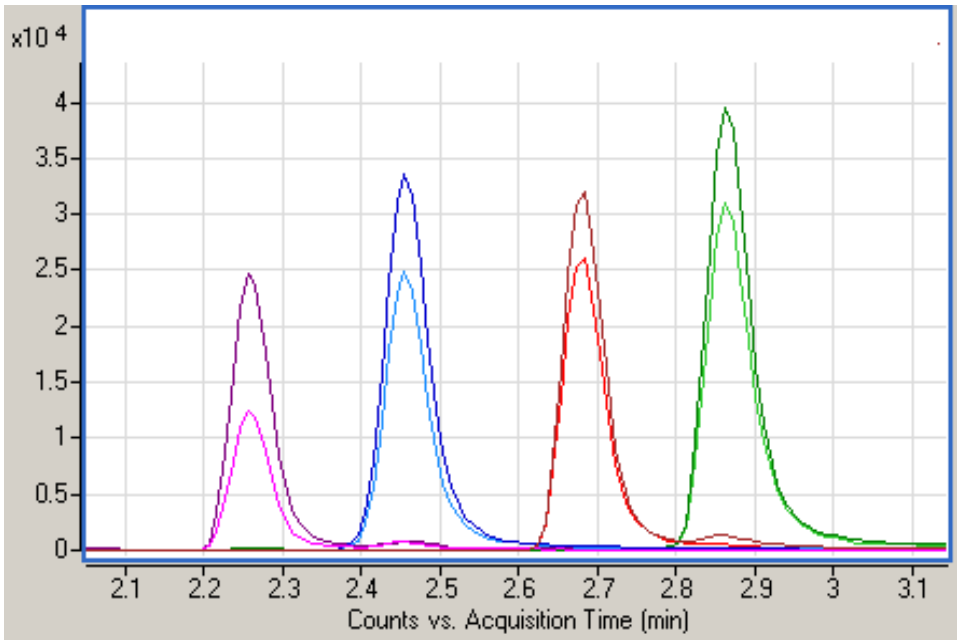
- Reasons
  - screening of wide range of co-occurring analytes necessary for evaluation of synergistic effects of different mycotoxins
  - identification of mycotoxins in unlikely matrices
  - monitoring of changes in regional fungal spread (climate change)
  - analysis of food and feed mixtures of several commodities

- HR-MS/MS vs QqQ
  - QTOF: target and non-target analysis
    + number of analytes
    + unknown contaminants
    + post-acquisition data analysis
    - higher LODs compared to QqQ
  - QqQ: target analysis
    + low LODs
    - lower mass accuracy
    - dwell time limited

QqQ

MRM vs. Dynamic MRM





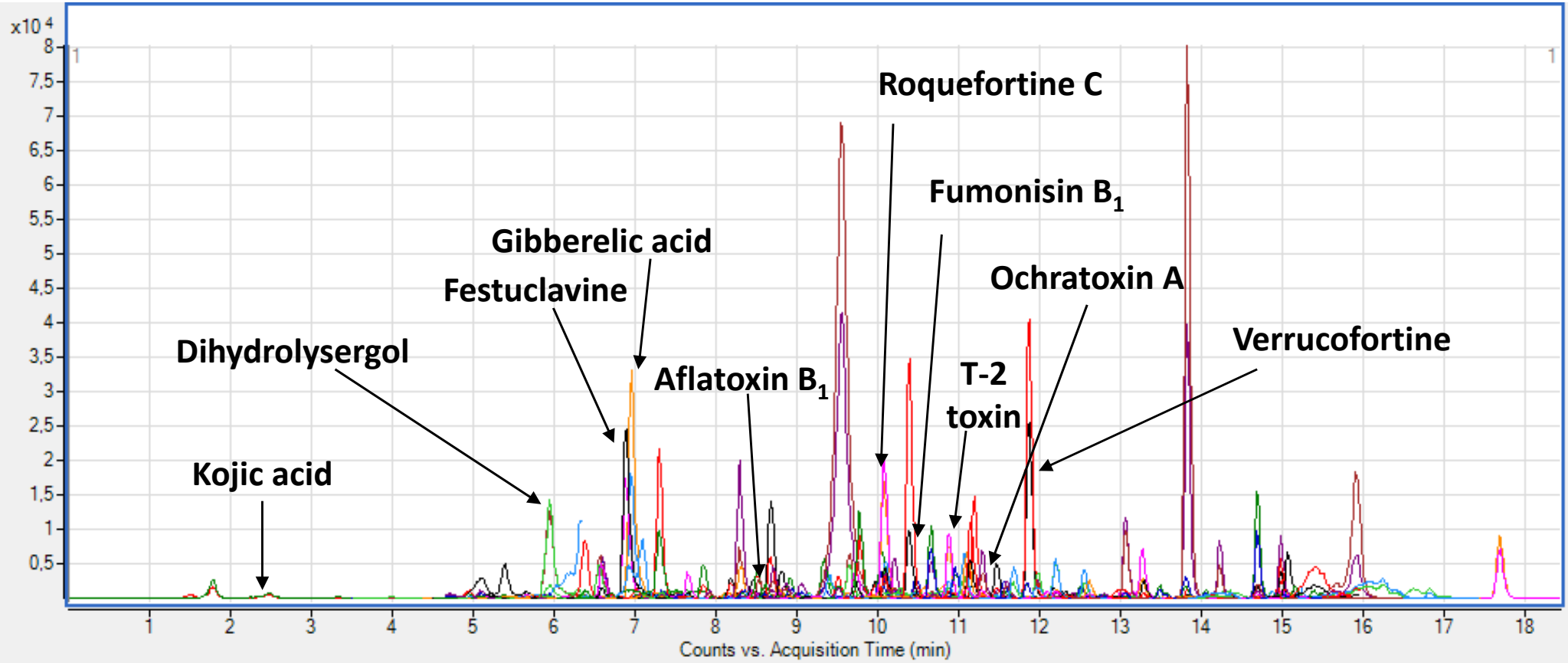
static (classical) MRM

Dynamic MRM

UHPLC-QqQ

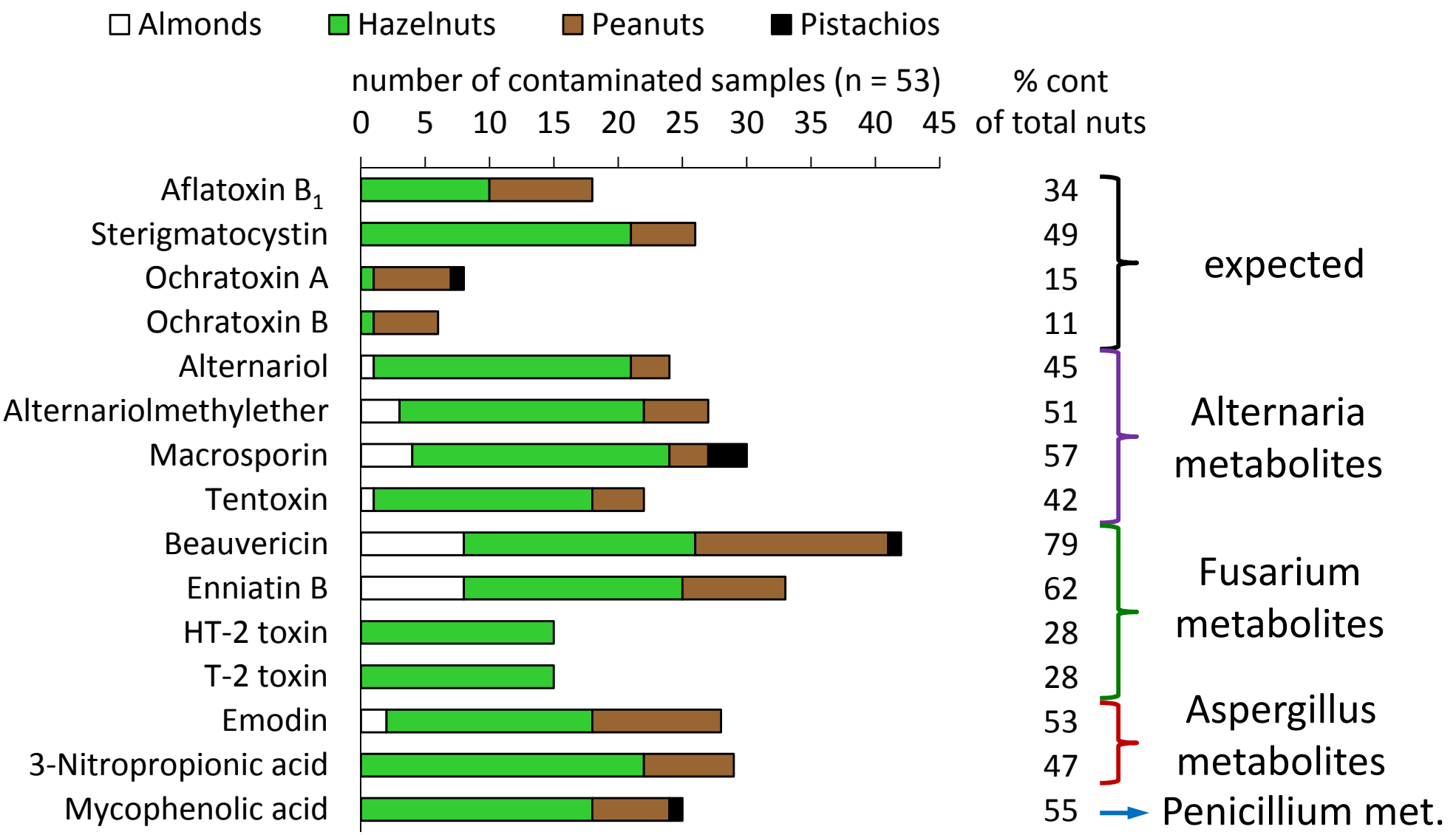
Multi-toxin Screening

- (semi-)quantitative method, validated for nuts (6460 QqQ MS)
- + MRM of a spiked hazelnut sample (medium level)
  191 mycotoxins and other fungal metabolites



Market survey

Nuts

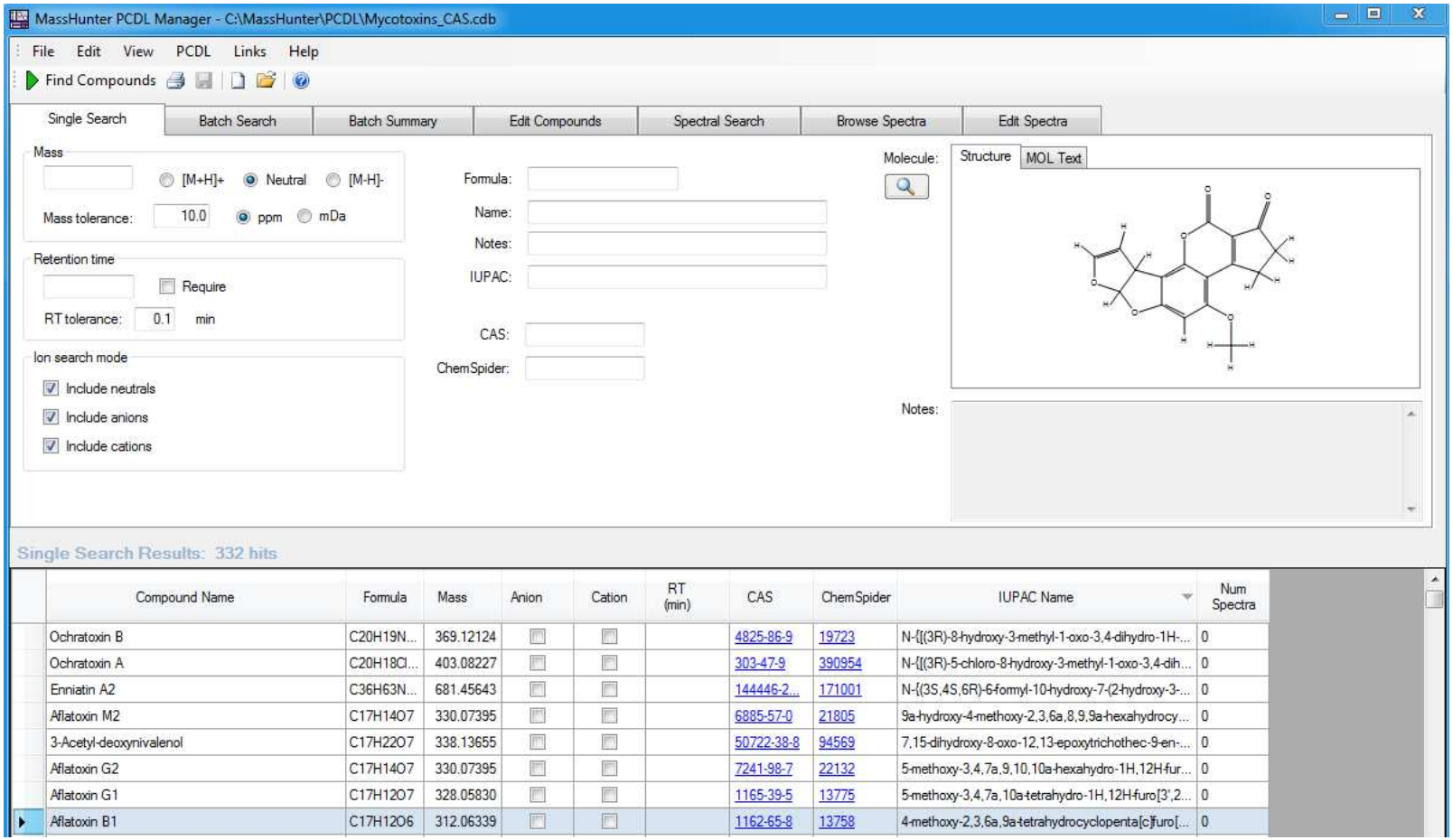


QTOF

Mycotoxin-Screening

Personal Compound Database and Library

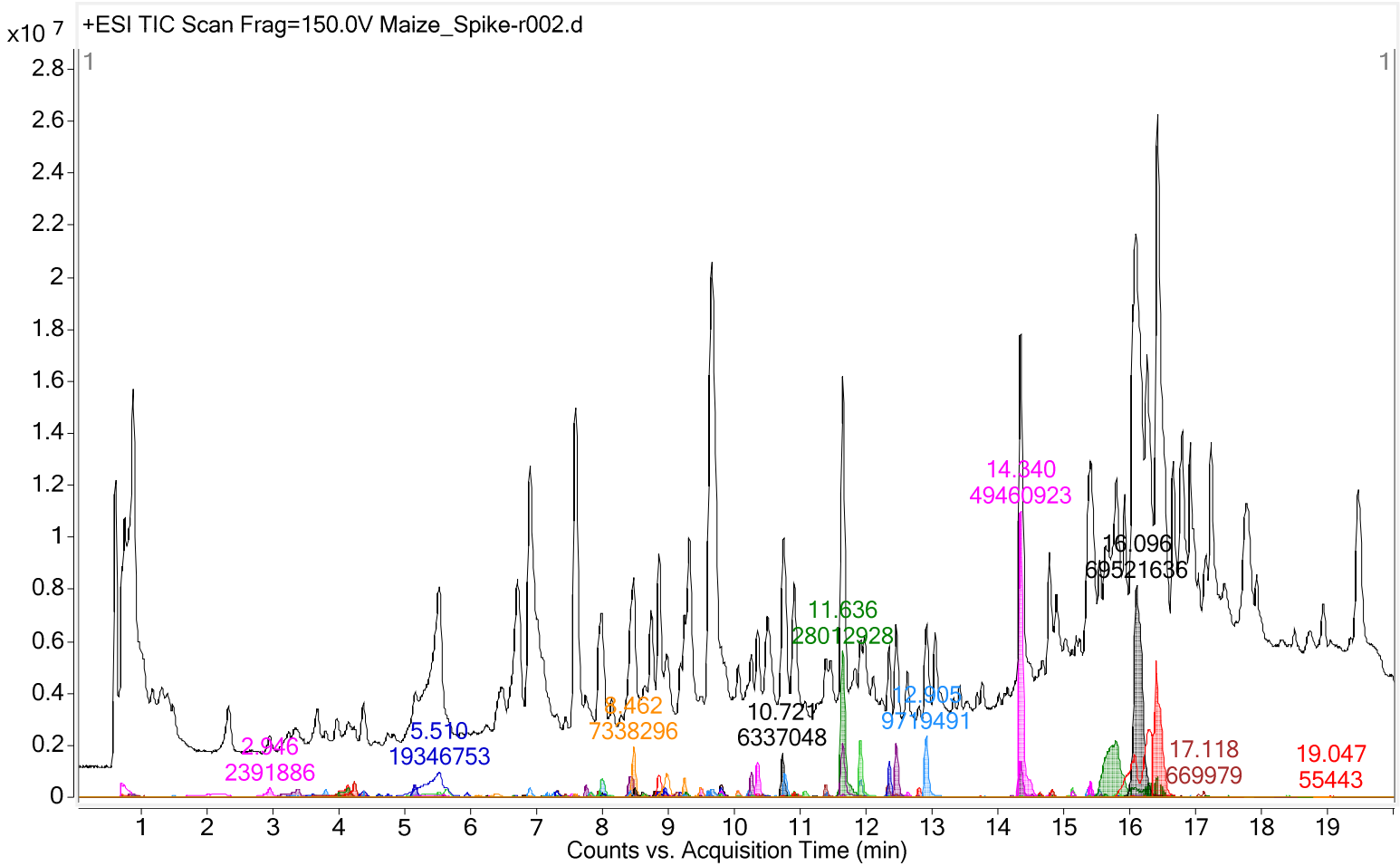
- Containing mycotoxins and fungal metabolites



QTOF

Example

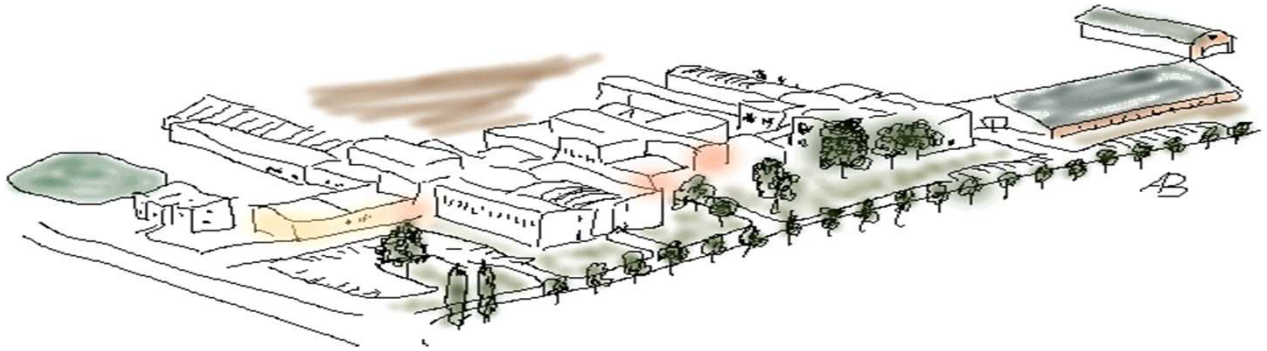
- 80 common mycotoxins and fungal metabolites spiked in maize extract to 1 to 5000 ng/mL



Summary

Screening methods

- for mycotoxins using QqQ or QTOF
- (semi-)quantitative determination
- QqQ: paper submitted to Anal Bioanal Chem
- QTOF: creation of HR-MS/MS spectral database



Thank you for your attention!

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