



Mycotoxin analysis in cereal foods by collision cell fragmentation – high resolution mass spectrometry

Veronica M.T. Lattanzio

*National Research Council of Italy
Institute of Sciences of Food Production*



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Istituto Zooprofilattico delle Regioni Lazio e Toscana (Roma)



MYCOTOXINS

- ✓ **Secondary metabolites** produced by filamentous fungi
- ✓ **Low-molecular-weight natural products**
- ✓ **Chemically stable compounds**
- ✓ **Toxic compounds** for human and animals (acute and chronic toxicity)



Principal mycotoxins and relevant producer fungi

MICOTOXIN	COMMODITY	FUNGAL PRODUCERS
Aflatoxins B1, B2, G1, G2	Corn, peanuts, spices, dried fruits	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Aflatoxin M1	Milk, eggs, cheese	
Ochratoxin A	Wheat, barley, corn, coffee, wine, beer	<i>Aspergillus ochraceus</i> , <i>A. carbonarius</i> , <i>A. niger</i> , <i>Penicillium verrucosum</i>
Trichothecenes (DON, T-2, HT-2)	Wheat, corn, oats, barley	<i>Fusarium graminearum</i> , <i>F. culmorum</i> , <i>F. poae</i>
Zearalenone	Corn, wheat	<i>Fusarium graminearum</i> , <i>F. culmorum</i> , <i>F. crookwellense</i>
Fumonisin	Corn, corn based-product	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>



Fungal Ecology and Mycotoxin Production in Food



planting



harvest



transport



storage

transport

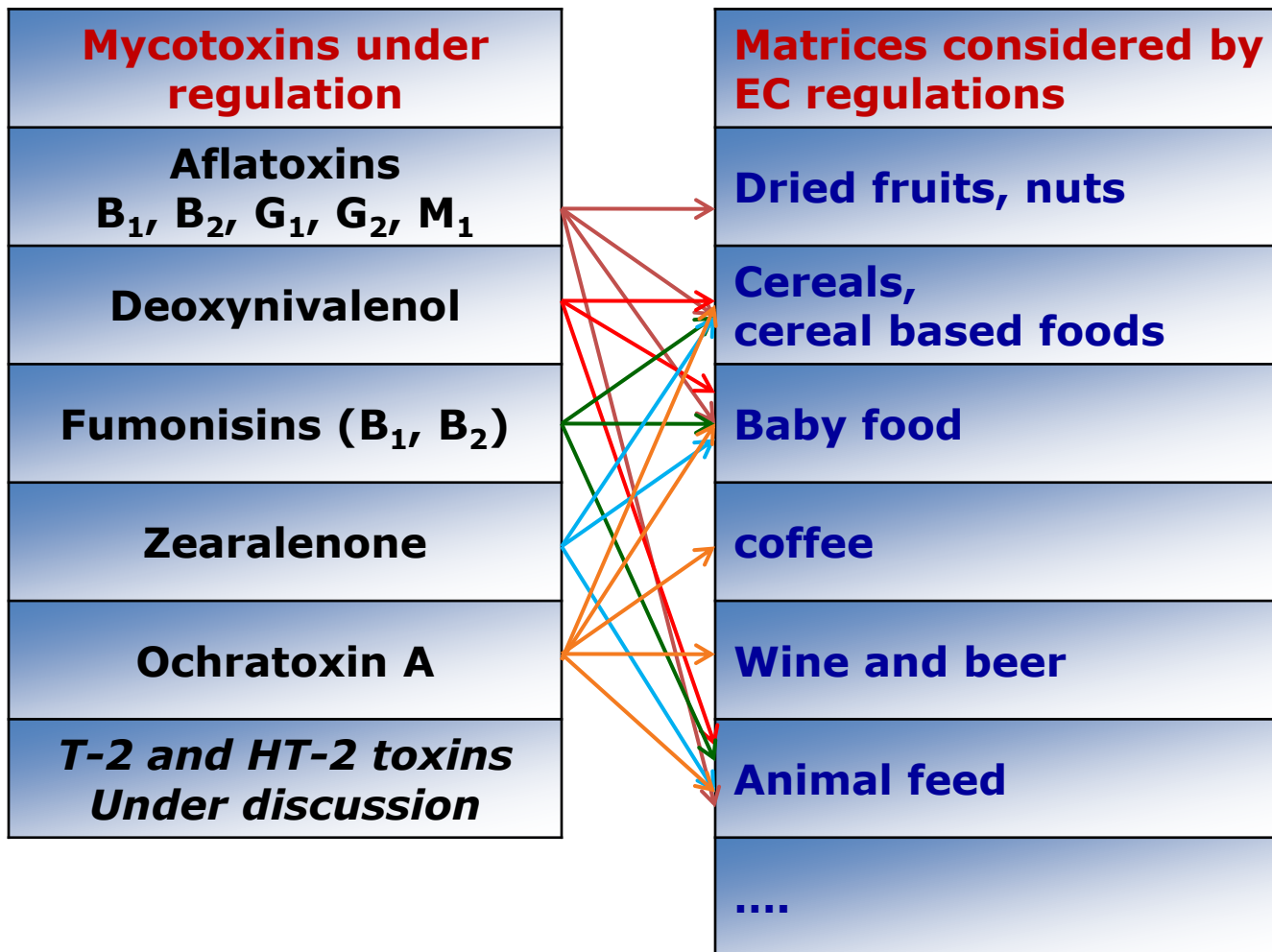


processing

distribution



Need to improve **mycotoxin monitoring and prevention to minimize contamination at different critical steps of the food chain "from farm to fork"**



Maximum levels
range from:
ng/kg to **mg/kg**

Need of **reliable** analytical methods
applicable at regulatory levels
in a **wide range** of matrices

- **Official control**
- **Monitoring**
- **Risk assessment**


RASFF: Rapid Alert System for Food and Feed

(http://ec.europa.eu/food/food/rapidalert/index_en.htm)

Total notifications: 3812

2nd - Mycotoxins: 635

1st - Pathogenic micro-organisms : 599



product category	aflatoxins	DON	fumonisin	ochratoxin A
cereals and bakery products	13	11	4	5
feed	119			1
fruits and vegetables	78			10
herbs and spices	51			17
nuts, nut products and seeds	320			
other	4			2
total	585	11	4	35



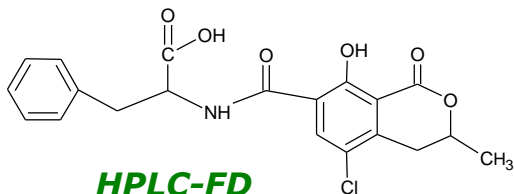
Presentation Outline

Explore the use of **Orbitrap™** based HRMS for the **quantitative** determination of **regulated mycotoxins** in **small cereal grains and related foods** and for identification and characterization of mycotoxin-related compounds.

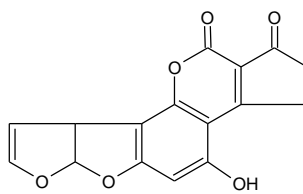
1. Development of an LC-HCD-HRMS method for the simultaneous determination of **aflatoxins (B₁, B₂, G₁, G₂), ochratoxin A, deoxynivalenol, zearalenone, T-2 and HT-2 toxins**. Use and optimization of **HRMS** conditions to obtain **quantitative** and **confirmatory** information.
2. Evaluation of method **performances** and comparison between the **LC-HCD-HRMS** and **LC-MS/MS** (triple quadrupole) detection approaches.
3. Approaches to manage **matrix effects** in LC-MS mycotoxin analysis.
4. Use of **HRMS for metabolite discovery**

Mycotoxin analysis

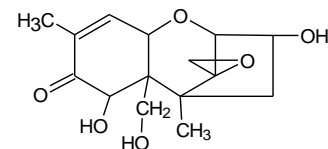
OCHRATOXIN A



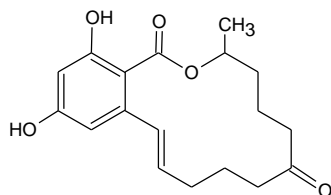
AFLATOXIN B₁



DEOXYNIVALENOL



ZEARALENONE



Different chemical nature:

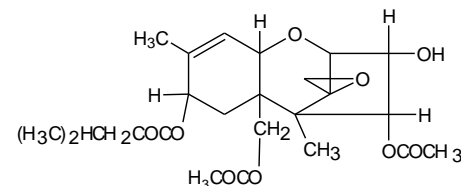
- Polarity
- UV absorption or fluorescence
- Ionic form (pH dependent)

Different target levels
(from µg/kg up to mg/kg)

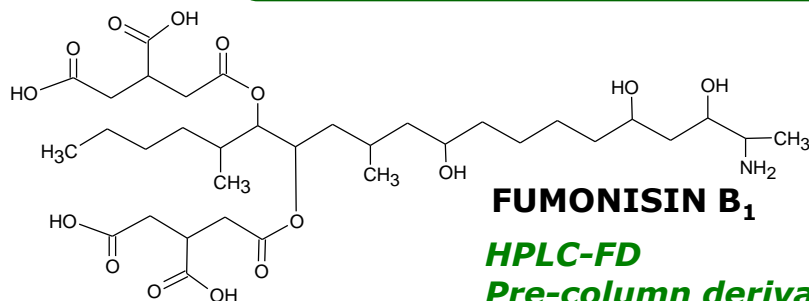
Presence in different matrices

HPLC-FD
Post column derivatization

T-2 TOXIN



Different strategies for sample preparation and analysis



**Multi-mycotoxin analysis
poses challenges in each
step of method development**

Sample Preparation

Critical points in sample preparation

⇒ Quantitative co-extraction of all concerned analytes

High methanol or acetonitrile percentages ($> 75\%$)

AFs, OTA, ZEA, DON, T-2/HT-2

High water content (50%) or low pH

Fumonisin

⇒ Analyte purification



Immunoaffinity columns

Solid phase extraction

(polymeric columns, adsorbents...)

QuEChERS

Dilute and shoot



Analytical tools { **LC-MS/MS** ⇒ quantitative analysis
LC-HRMS ⇒ qualitative analysis/screening

Number of mycotoxins-matrix	Extraction solvent - cleanup	LOD range (µg/kg)	Recovery range (%)	Type of LC-MS detection	References
13 mycotoxins in corn, wheat, cornflakes, biscuits.	Acetonitrile/water (85:15) Multisep #226	Wheat: 0.1-3.8 Corn: 0.1-4.9	Wheat: 71-132 Corn: 81-133	LC-APCI-TOFMS	Tanaka et al. 2006
39 mycotoxins in wheat and maize.	Acetonitrile/water/ acetic acid (79:20:1) Direct injection	Wheat:0.03-170 Maize:0.03-220	Wheat:43-104 Maize:57-110	LC-ESI-MS/MS (MRM mode)	Sulyok et al. 2006
87 mycotoxins in bread crumbs.	Acetonitrile/water/ acetic acid (79:20:1) Direct injection	0.03-225	59-114	LC-ESI-MS/MS (MRM mode)	Sulyok et al. 2007
17 mycotoxins in peanuts butter	Acetonitrile/water (84:16) Mycosep 226 + Aflazon+	0.003-0.212	70.6-119	UPLC-ESI-MS/MS (MRM mode)	Ren et al. 2007
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		Maize:0.4-2000 Wheat:0.4-200	Maize:68-152 Wheat:87-131	LC-ESI-LTQ-Orbitrap	
31 mycotoxins in wheat, barley and oats	Acetonitrile/water (90:10) Direct injection	Wheat:0.5-300	Wheat:7-140	LC-ESI-MS/MS (MRM mode)	Kokkonen et al. 2009
12 mycotoxins in maize, walnuts, biscuits and breakfast cereals.	acetonitrile/water (80:20) Direct injection	Maize:0.01-1.50	Maize:71.1-92.1	UPLC-ESI-MS/MS (MRM mode)	Garrido-Frenich et al.2009
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11 mycotoxins in maize, wheat, barley	Water 0.1% formic acid/acetonitrile (QuEChERS)	Maize:5-50	Maize: 43-120	UPLC-ESI-TOF-MS	Zachariasova et al. 2010
	Water 0.1% formic acid/acetonitrile (1:1) Direct injection	Maize:10-30	Not tested	UPLC-APCI-OrbitrapMS	
17 mycotoxins in cereal flours and cereal foods	Water/MeCN 0.5% acetic acid (1:1) (QuEChERS)	0.5-100 (LOQ)	Maize flour: 58-108	LC-ESI-MS/MS (MRM mode)	Desmarchelier et al. 2010
	MeCN/water/glacial acetic acid (80:19:0.5) (QuEChERS)		Maize flour: 77-97		
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Comparison of MS/MS and HCD-HRMS approaches

V.M.T. Lattanzio, S. Della Gatta, M. Godula, A. Visconti. *Food Additives and Contaminants*, 2011, 28(10):1424-37.



Wheat flour

Barley flour

Oat flour



Wheat based crisp bread
Rye based crisp bread

Breakfast cereals
Cereal biscuits



Sample



Extraction

acetonitrile/water
(84/16)



Filtration (Whatmann N°4)

Evaporation

Reconstitution in methanol/water (10/90)

Clean-up with

OASIS® HLB

(polimeric SPE cartridges)

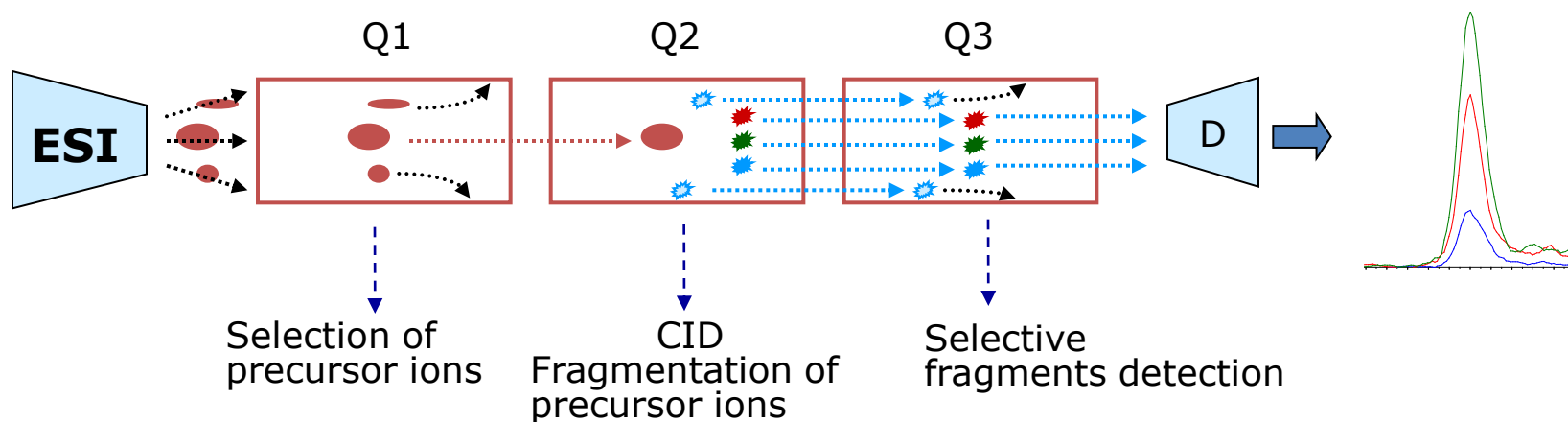
LC-MS/MS analysis

LC-HRMS analysis

LC-MS/MS analysis

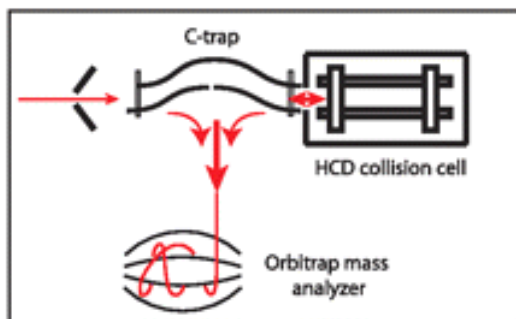
Triple Quadrupole Tandem MS

Multiple Reaction Monitoring

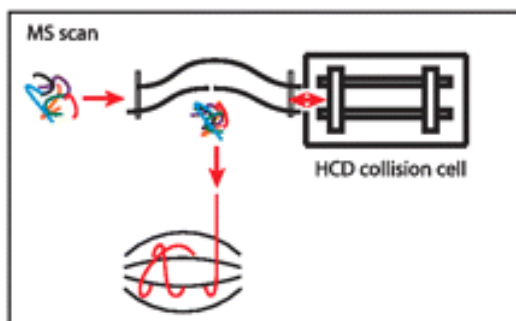


LC-HCD-HRMS analysis

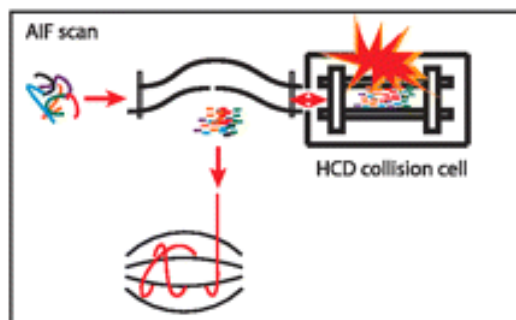
Exactive™ : HCD – Orbitrap



*This instrument, termed Exactive™, is capable of generating **"all ion fragmentation"** in a nonselective manner using a collision cell **without precursor ion selection**.*

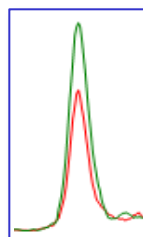
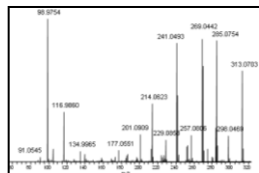
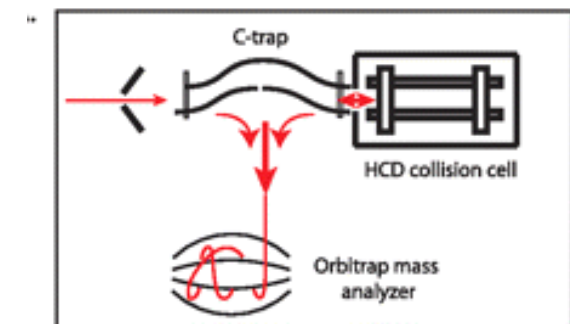


➔ **MS scan mode** for precursors: electrosprayed ions are accumulated in the C-trap, then injected into the orbitrap analyzer.

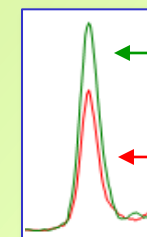
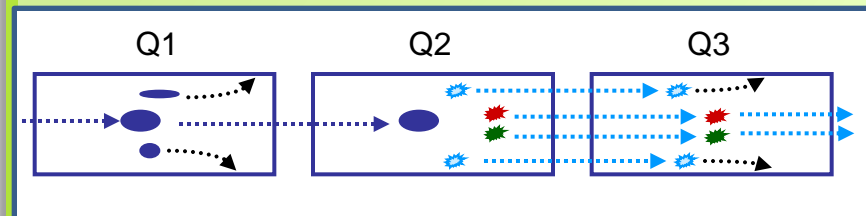


➔ **All Ion Fragmentation mode:** all electrosprayed ions are fragmented by collisions with nitrogen gas in the HCD cell. Fragments are then moved back to the C-trap from where they are injected into the orbitrap analyzer.

Comparison of MS/MS and HCD-HRMS approaches



Full scan
HCD fragmentation
Mass accuracy up to 0.5 ppm



Quantifier ion

Qualifier ion

Selectivity

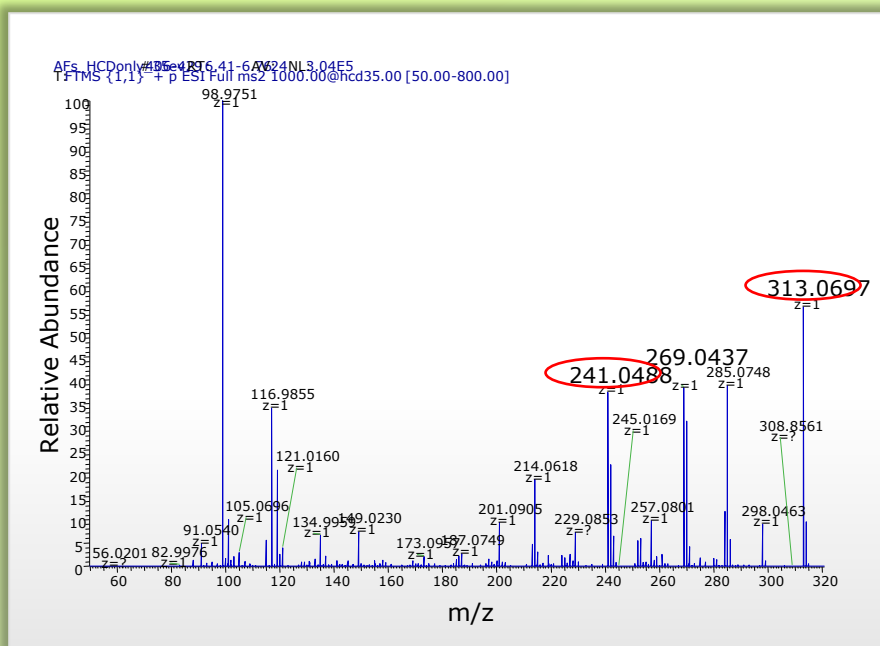
Multiple Reaction Monitoring
Mass Accuracy > 500 ppm

MS/MS detection HRMS detection legislation requirements

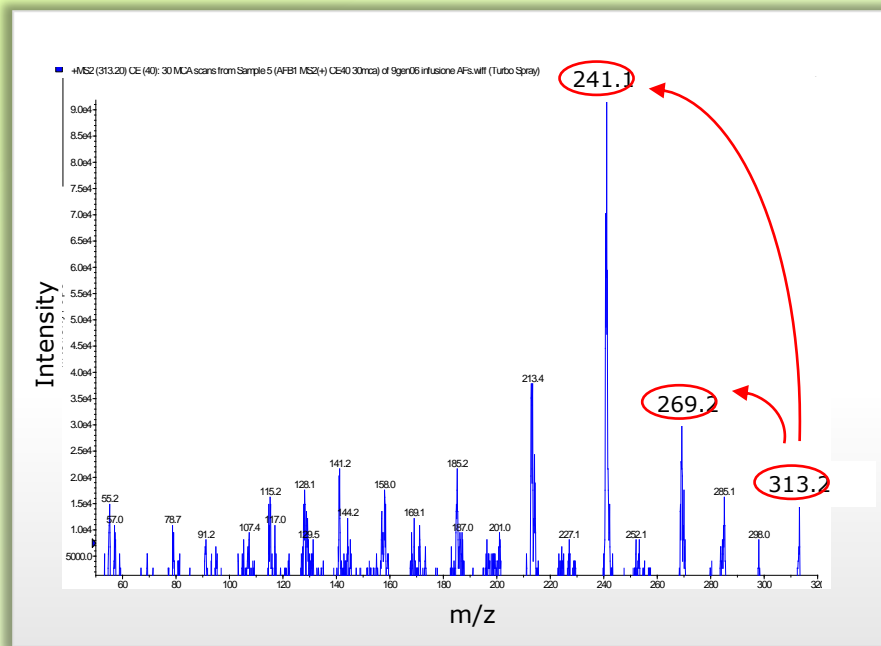
***EC performance criteria (2002/657/EC)
Doc No Sanco/10684/2009****

MS/MS	HCD-HRMS
1 precursor ion 2 daughters ions	2 ions Mass accuracy < 5ppm*
<i>example: AFB1</i>	
313.2 – 269.2	313.0707*
313.2 – 241.1	241.0495

HCD-HRMS spectrum of standard AFB₁ (collision energy 35 eV)

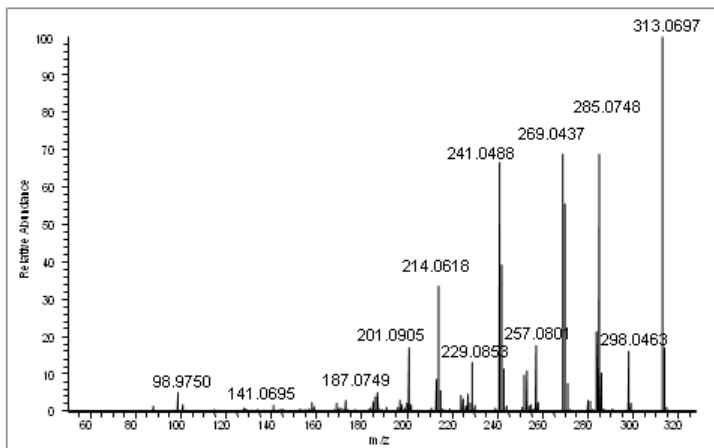


MS/MS spectrum of standard AFB₁ (collision energy 40 eV)

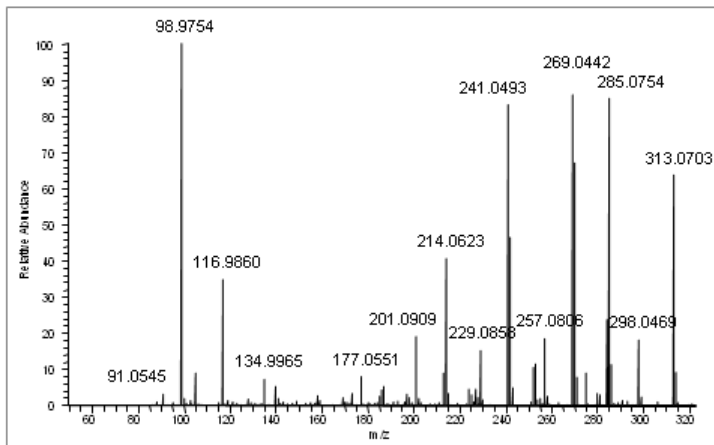


Similar fragmentation patterns can be obtained by using MS/MS or HCD-HRMS fragmentation

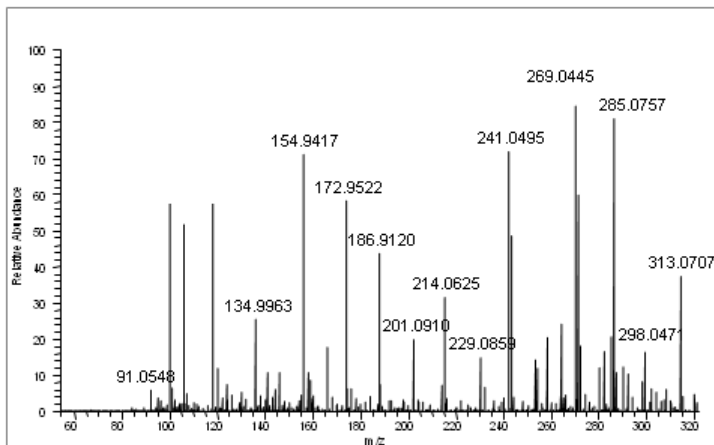
LC-HCD-HRMS spectra in food extracts Aflatoxin B₁



standard
solution



Barley flour
extract



Wheat flour
extract

**Main characteristic
fragments can be easily
detected in food extracts.**



Mass Accuracy

in standard solution and cereal food extracts

Mycotoxin	Calculated mass	Mass accuracy (ppm)*			
		Standard solution	Wheat flour	Barley flour	Crisp bread (rye based)
DON	297.13381	2.2	0.2	0.6	3.9
	231.10157	1.0	0.4	0.3	1.9
AFG ₂	331.08123	0.9	0.3	1.7	0.8
	245.30808	0.5	0.6	1.1	1.5
AFG ₁	329.06558	1.2	0.2	1.5	1.8
	243.06518	1.2	0.5	1.3	1.3
AFB ₂	315.08631	1.2	0.2	1.6	0.9
	287.09140	1.1	0.4	1.7	1.5
AFB ₁	313.07066	1.1	0.1	1.3	2.6
	241.04953	1.0	0.3	1.2	1.2
HT-2	442.24409	1.2	0.2	0.3	1.6
	245.11722	1.2	0.6	1.2	1.6
T-2	484.25460	0.7	0.4	1.7	1.9
	215.10660	0.4	0.1	0.5	1.6
ZEA	319.15450	1.0	0.1	0.6	2.2
	283.13287	0.9	0.4	2.1	1.5
OTA	404.08950	0.8	0.1	0.5	1.6
	358.08406	1.0	0.3	0.8	1.5

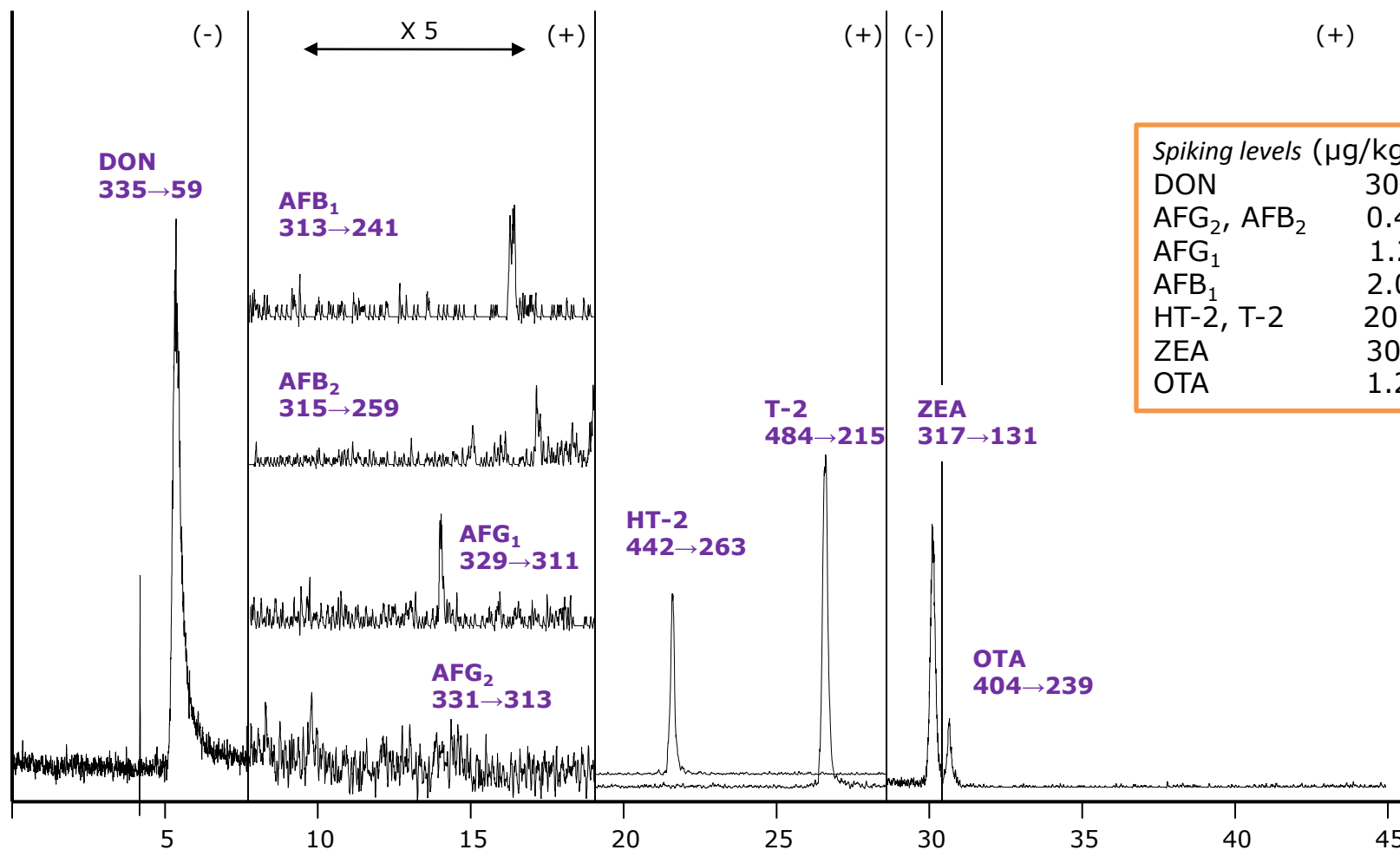
* Absolute value, average of triplicate injections of 1 ng toxin

MRM method for mycotoxin detection selected ions in Q1 and Q3

Mycotoxin	Precursor ion	Q1 (m/z)	Q3 (m/z)
DON	[DON+CH ₃ COO] ⁻	355.1	295.0
			59.0 *
AFG ₂	[AFG ₂ +H] ⁺	331.1	313.2 *
			245.3
AFG ₁	[AFG ₁ +H] ⁺	329.1	311.2
			243.1 *
AFB ₂	[AFB ₂ +H] ⁺	315.0	287.2
			259.0 *
AFB ₁	[AFB ₁ +H] ⁺	313.2	241.1 *
			213.4
HT-2	[HT-2+NH ₄] ⁺	442.0	263.2 *
			215.0
T-2	[T-2+NH ₄] ⁺	484.3	215.2 *
			185.3
FB ₁	[FB ₁ +H] ⁺	722.4	352.4
			334.5*
FB ₂	[FB ₂ +H] ⁺	706.4	336.6*
			318.3
OTA	[OTA+H] ⁺	404.0	239.0 *
			221.1
ZEA	[ZEA-H] ⁻	317.0	175.1
			130.9 *

→ **Identity confirmation**
→ **Quantification**

LC-MS/MS chromatogram of a spiked wheat flour extract



Spiking levels (µg/kg)	
DON	300
AFG ₂ , AFB ₂	0.4
AFG ₁	1.2
AFB ₁	2.0
HT-2, T-2	20
ZEA	30
OTA	1.2

Column: Gemini RP18 (150 × 2.0 mm, 5 µm) Phenomenex
Flow: 200 µl/min
Column oven: 40 °C
Solv A: H₂O, 0.5% acetic acid, 1mM AcNH₄
Solv B: CH₃OH, 0.5% acetic acid, 1mM AcNH₄
Injection volume: 20µl (100 mg sample)

HCD-HRMS method for mycotoxin detection

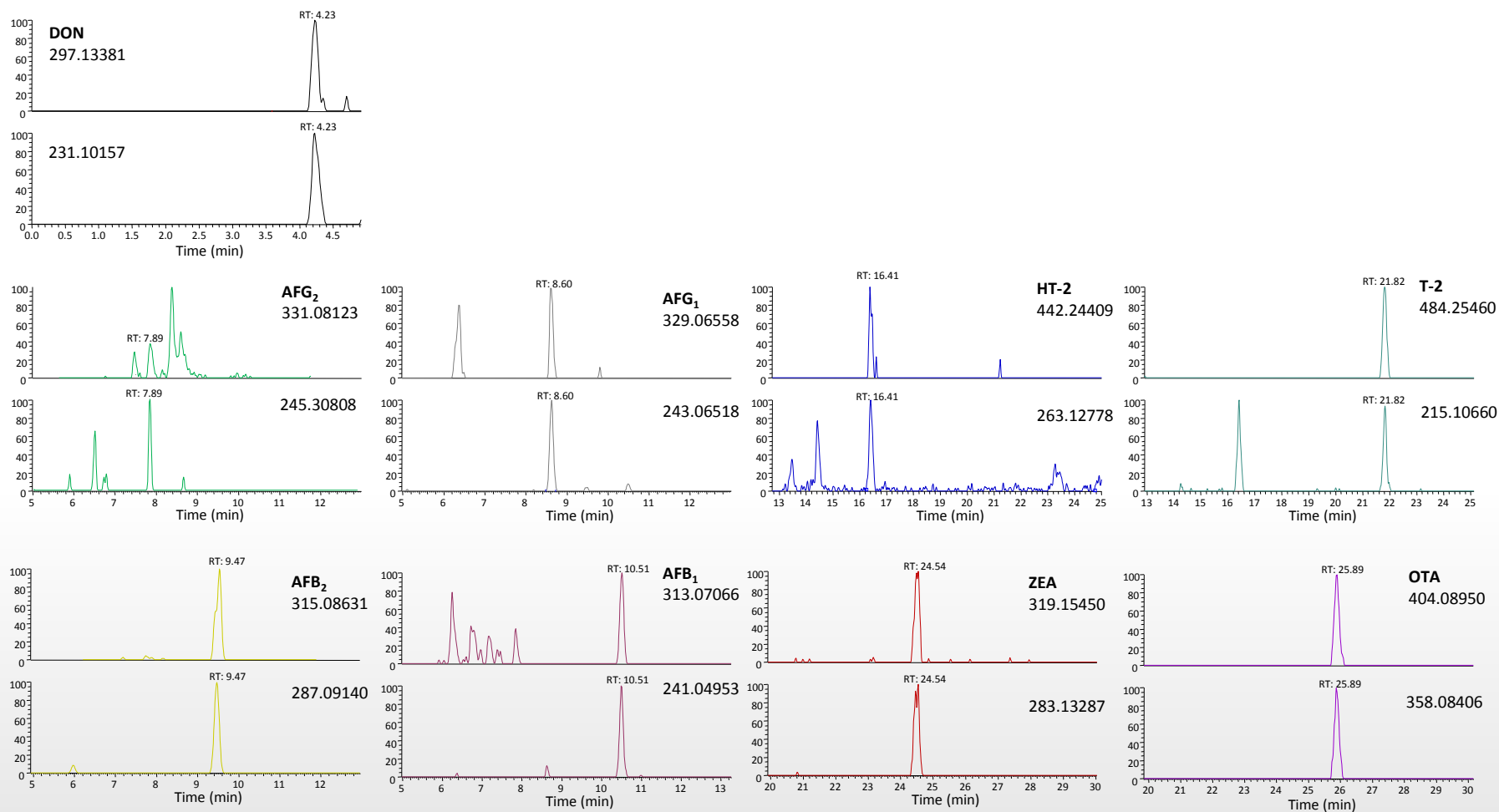
Ion extracted from the full scan chromatogram

Mycotoxin	Molecular formula	Calculated mass	HCD (eV)
DON [M+H] ⁺	C ₁₅ H ₂₁ O ₆	297.13381	15
	C ₁₄ H ₁₅ O ₃	231.10157*	
AFB₁ [M+H] ⁺	C ₁₇ H ₁₃ O ₆	313.07066	35
	C ₁₄ H ₉ O ₄	241.04953*	
AFB₂ [M+H] ⁺	C ₁₇ H ₁₅ O ₆	315.08631	35
	C ₁₆ H ₁₅ O ₅	287.09140*	
AFG₁ [M+H] ⁺	C ₁₇ H ₁₃ O ₇	329.06558	35
	C ₁₄ H ₁₁ O ₄	243.06518*	
AFG₂ [M+H] ⁺	C ₁₇ H ₁₅ O ₇	331.08123	35
	C ₁₄ H ₁₃ O ₄	245.30808*	
HT-2 [M+NH ₄] ⁺	C ₂₂ H ₃₆ NO ₈	442.24409	10
	C ₁₅ H ₁₇ O ₃	245.11722*	
T-2 [M+NH ₄] ⁺	C ₂₄ H ₃₈ NO ₉	484.25460*	10
	C ₁₄ H ₁₅ O ₂	215.10660	
ZEA [M+H] ⁺	C ₁₈ H ₂₃ O ₅	319.15450*	10
	C ₁₈ H ₁₉ O ₃	283.13287	
OTA [M+H] ⁺	C ₂₀ H ₁₉ NO ₆ Cl	404.08950*	10
	C ₁₉ H ₁₇ NO ₄ Cl	358.08406	

→ Identity confirmation

→ Quantification

LC-HCD-HRMS chromatogram of a spiked wheat flour extract



Spiking levels: 25 µg/kg DON, AFG₁, AFB₁, T-2, HT-2, ZEA, OTA and 6.2 µg/kg AFG₂, AFB₂

RECOVERIES and REPEATABILITY

EC acceptance criteria (401/2006/EC)

		Recoveries, % (RSDr %)								
		DON	AFG ₂	AFG ₁	AFB ₂	AFB ₁	HT-2	T-2	ZEA	OTA
Spiking levels (µg/kg)		300	0.4	1.2	0.4	2	20	20	30	1.2
Wheat flour	HCD- HRMS	102 (5)	90 (8)	89 (0)	95 (2)	81 (6)	104 (4)	98 (6)	76 (6)	97 (9)
	MRM	95 (2)	n.d.	82 (4)	84 (6)	89 (4)	95 (4)	92 (4)	95 (9)	74 (7)
Crisp bread (wheat)	HCD- HRMS	104 (0)	102 (5)	104 (4)	80 (2)	102 (2)	105 (1)	103(1)	85 (1)	93 (2)
	MRM	100 (0)	n.d.	106 (5)	85 (10)	102 (6)	107 (2)	108 (6)	84 (5)	101 (3)
Crisp bread (rye)	HCD- HRMS	105 (1)	93 (2)	95 (6)	93 (8)	87 (4)	100 (3)	95 (3)	101 (9)	74 (13)
	MRM	95 (3)	91 (7)	79 (2)	85 (7)	77 (3)	97 (2)	91 (3)	96 (7)	82 (2)

DETECTION LIMITS

EC maximum permitted levels (1881/2006/EC)

	LOD (µg/kg)					
	Wheat Flour		Barley Flour		Crisp bread (rye)	
	HCD-HRMS	MRM	HCD-HRMS	MRM	HCD-HRMS	MRM
DON	1.6	3.9	1.8	10.3	2.3	59.2
AFG₂	1.5	0.1	0.5	0.2	0.5	1.9
AFG₁	0.6	0.2	1.1	0.7	1.2	2.6
AFB₂	0.7	0.3	0.5	0.3	0.5	1.1
AFB₁	1.0	0.3	1.0	0.5	1.6	1.1
HT-2	1.7	0.3	2.5	1.1	1.7	1.7
T-2	1.0	0.2	0.5	0.5	1.6	0.9
ZEA	1.0	2.8	1.4	4.0	2.3	5.8
OTA	1.4	0.1	1.9	0.3	2.9	0.4



Analysis of Reference Materials

Material description	Target analyte	Assigned value, µg/kg	Satisfactory range, µg/kg	Level of ¹³ C-IS addition, µg/kg	Results obtained by HRMS analysis, µg/kg
wheat flour FAPAS T2256	DON	774	517 - 1032	700	756 ± 113.4*
breakfast cereals FAPAS T2257	ZEA	69.5	38.9 - 100.1	100	48.6 ± 9.8*
maize FAPAS T04148	AFs	AFB ₁ 5.07	AFB ₁ 2.84 - 7.30	AFB ₁ 5.0	AFB ₁ 5.3 ± 1.3*
		AFB ₂ 1.06	AFB ₂ 0.60 - 1.53	AFB ₂ 2.0	AFB ₂ 1.0 ± 0.3*
		AFG ₁ 2.97	AFG ₁ 1.66 - 4.27	AFG ₁ 5.0	AFG ₁ 2.2 ± 0.8*
		AFG ₂ 1.25	AFG ₂ 0.70 - 1.80	AFG ₂ 2.0	AFG ₂ 1.2 ± 0.4*

* Maximum standard uncertainty calculated according to 401/2006/EC

The experimentally determined concentrations of target analytes were within the satisfactory range for all tested toxins

Quantitative multi-mycotoxin determination by LC-MS/MS and LC-HRMS CONCLUSIONS

- ✓ **Quantitative** and **confirmatory** information using HRMS (Orbitrap™ based high resolution with HCD fragmentation) or MS/MS (low resolution – triple quadrupole) can be obtained, meeting European regulatory requirements for confirmatory analysis.
- ✓ Both SPE/LC-MS based analytical procedures exhibited satisfactory recoveries and adequate detection limits to assess mycotoxin contamination in cereal foods at regulatory levels.



if proper calibration is applied

MATRIX EFFECTS

Matrix effects in mass spectrometry: co-eluting matrix interfering components can affect analyte ionization, resulting in signal enhancement or suppression.



Need for matrix assisted calibration for accurate quantitative analysis.



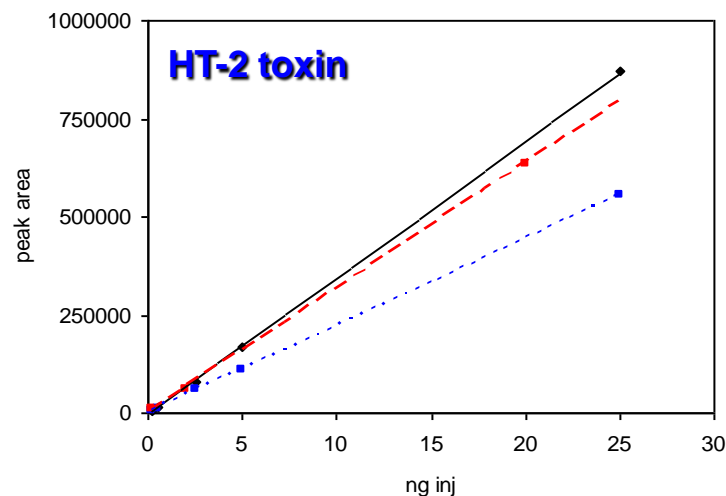
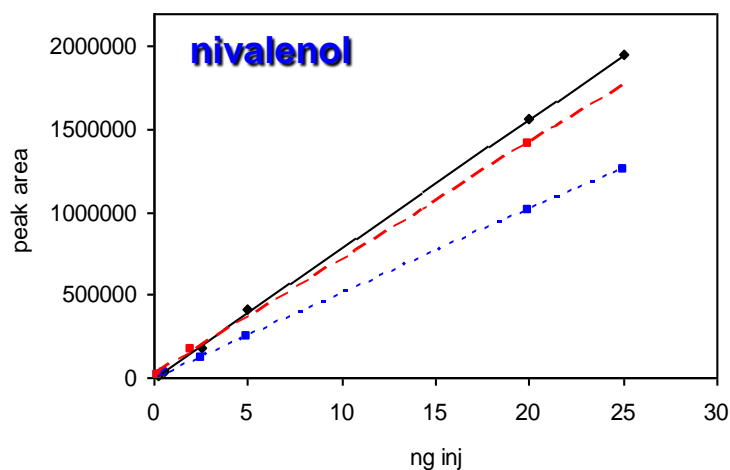
Different approaches:

- **External calibration** in a blank matrix
- Use of **isotope labelled** standards
- **Standard calibration** is sometimes used when the dilute & shoot approach is performed

Matrix Effect Evaluation

Comparison of calibration curves obtained by dissolving standard mycotoxins in:

- Mobile phase
- - - - - Semolina extract purified with **OASIS® HLB**
- Semolina extract purified with **Mycosep 227#**



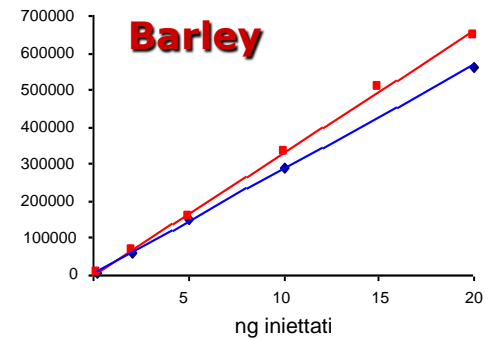
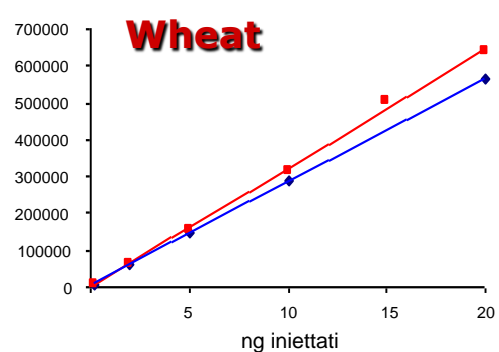
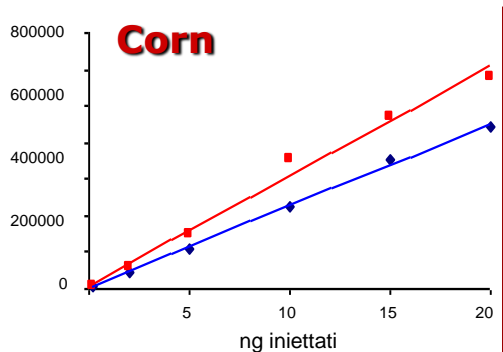
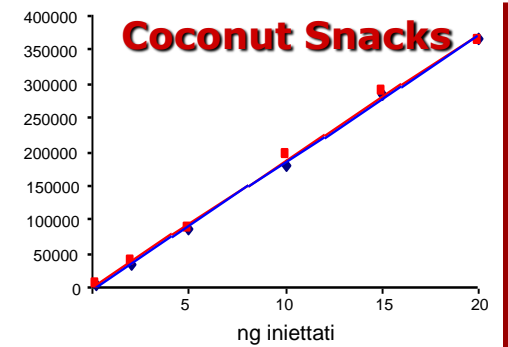
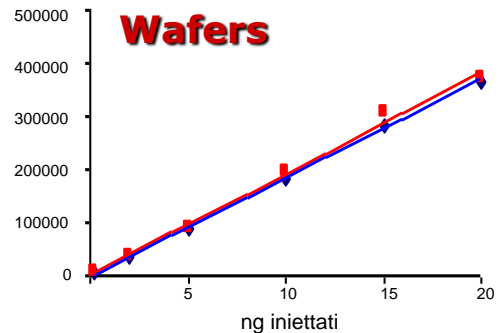
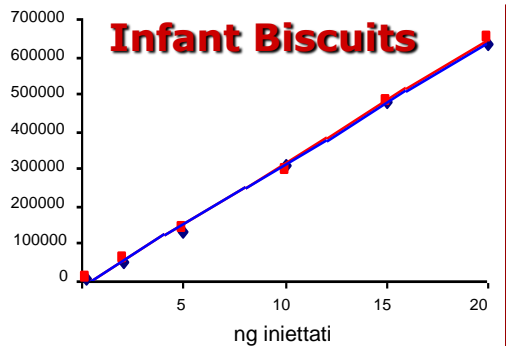
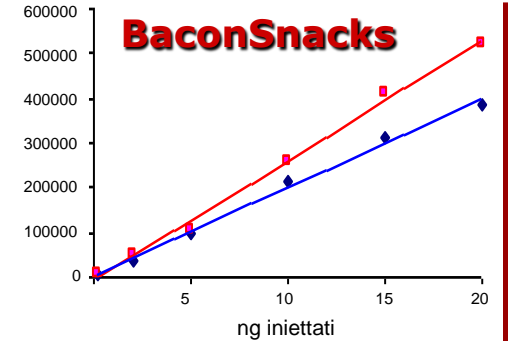
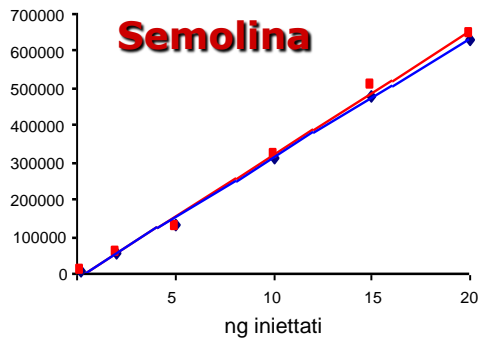
Improving sample preparation and column chromatography allows to minimize matrix effect.

Matrix Effect varies from matrix to matrix

T-2 toxin

extract purified
with OASIS HLB

mobile phase



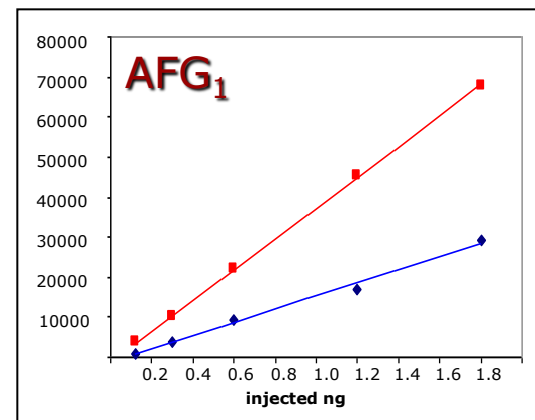
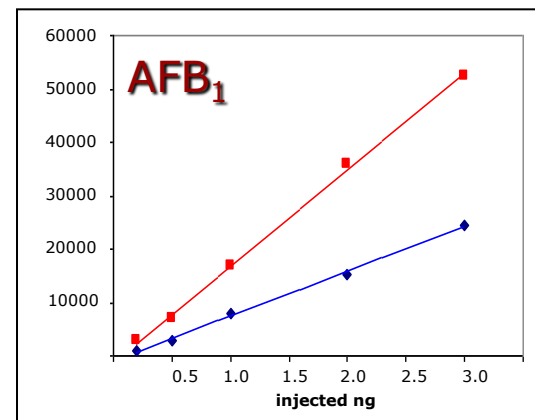
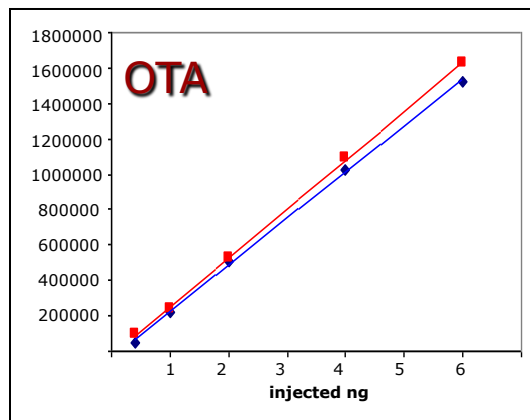
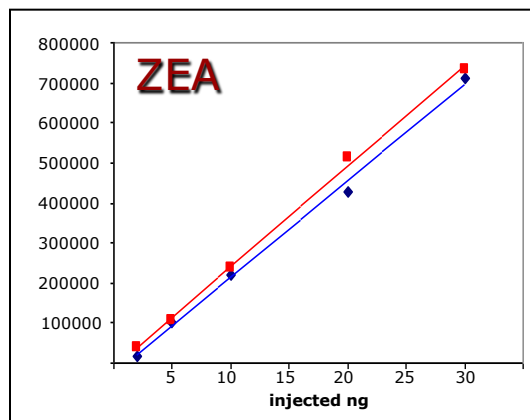
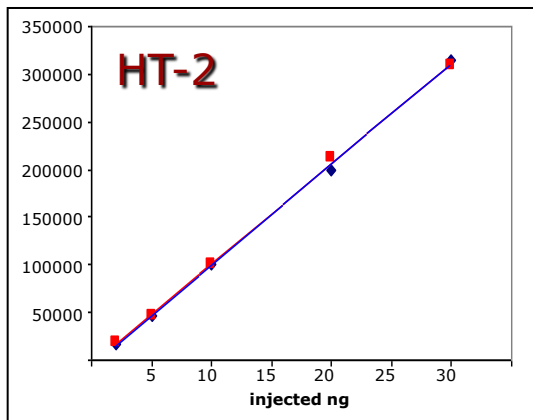
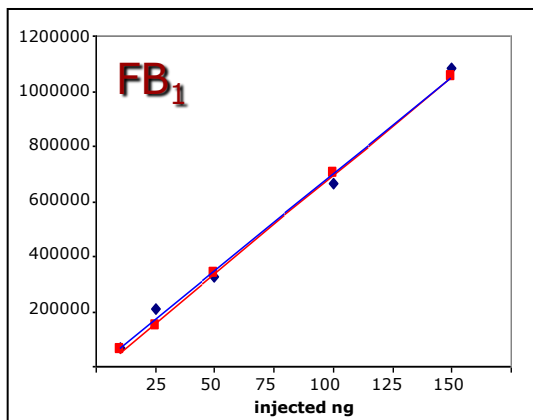
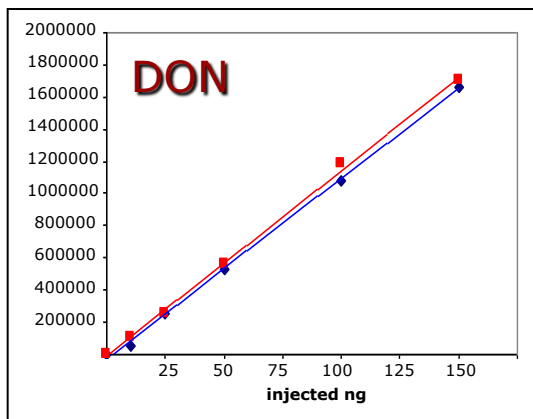
Matrix Effect varies from toxin to toxin

Calibrant solution obtained by dissolving standard toxins in:

— HPLC mobile phase

— Maize extract purified on Myco6in1 immunoaffinity columns

No matrix effect

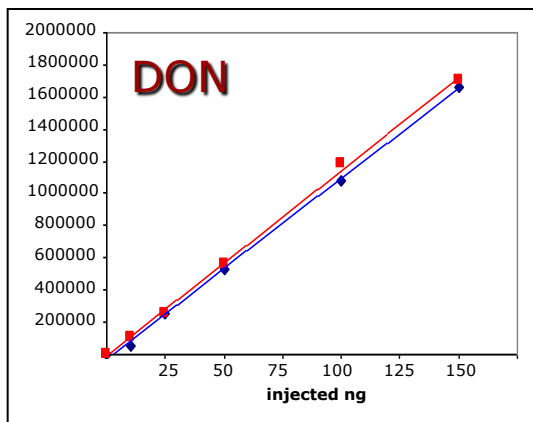


Ion suppression

Quantitative evaluation of Matrix effect (I)

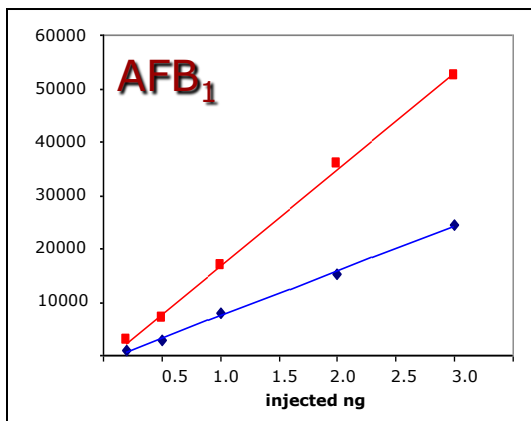
Calibrant solution obtained by dissolving standard toxins in:

- HPLC mobile phase
- Maize extract purified on Myco6in1 immunoaffinity columns – MRM detection



No matrix effect

Ion suppression



Statistical evaluation of the slope difference between standard and matrix-matched calibration curves

***t*-Test** ($p = 0.05$, $n = 6$, t value = 2.4)

	MAIZE	WHEAT	BARLEY
DON	-0.3	0.3	-0.1
AFG ₁	-6.2	2.2	2.0
AFB ₁	-15.0	0.2	-0.4
FB ₁	0.3	-	-
FB ₂	-1.6	-	-
HT-2	-0.5	0.6	0.6
T-2	-0.8	0.6	1.0
ZEA	-1.0	0.0	-6.8
OTA	-2.6	0.8	-0.6

Quantitative evaluation of Matrix effect (II)

SSE = signal suppression/enhancement
= (slope of matrix calibration/slope of standard calibration)*100

Comparison between calibration curves in:

- HPLC mobile phase
- Barley flour extract purified on SPE columns

Mycotoxin	Calibration range (µg/kg)	SSE, %	
		HCD-HRMS	MRM
DON	50-2500	76	95
AFG ₂	0.25-12.5	100	95
AFG ₁	1-50	100	89
AFB ₂	0.25-12.5	67	88
AFB ₁	1-50	100	88
HT-2	10-500	90	95
T-2	10-500	94	103
ZEA	10-500	65	70
OTA	1-50	87	106

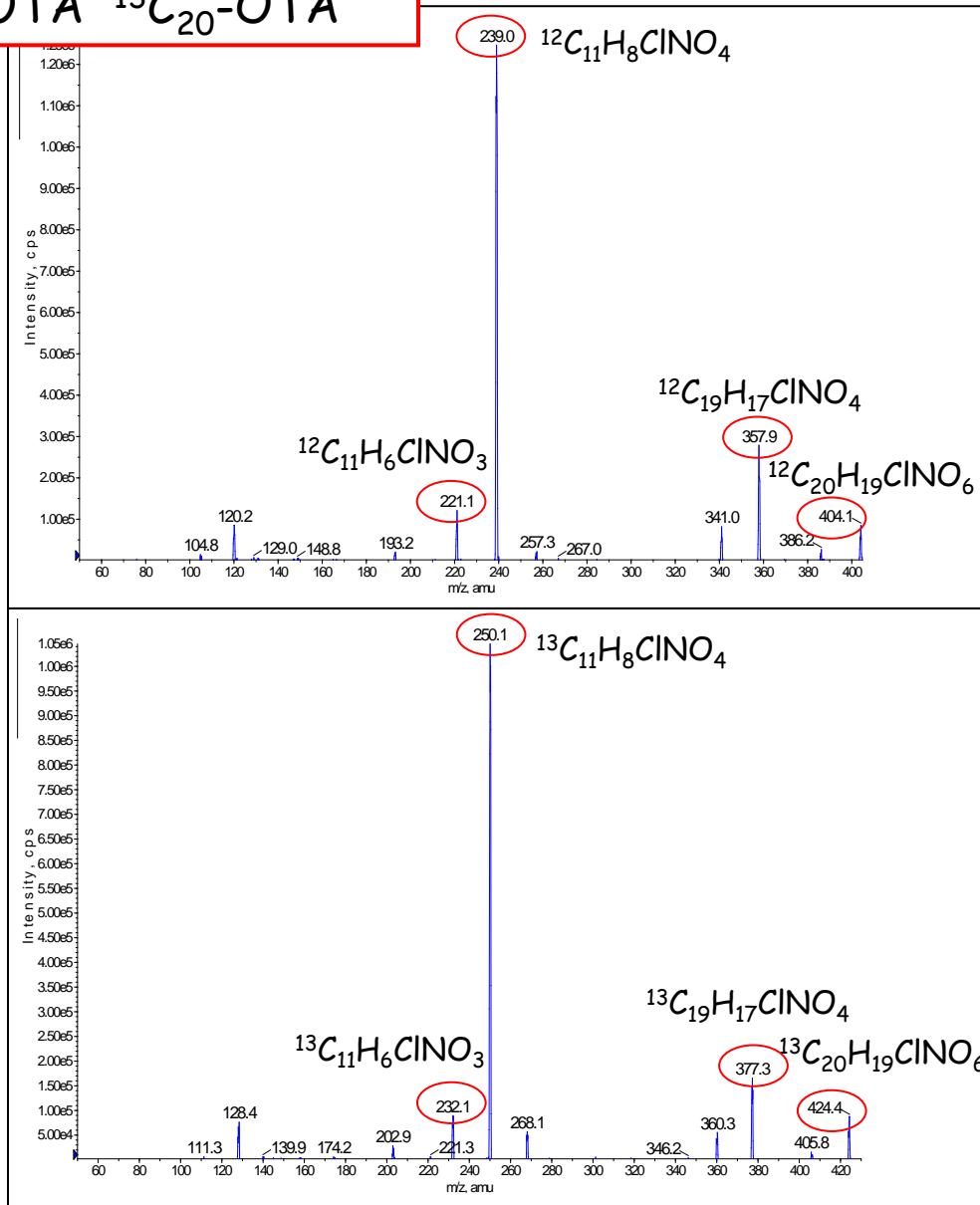
^{13}C labelled mycotoxins as internal standards

$^{12}\text{C}_{20}\text{-OTA}$ $^{13}\text{C}_{20}\text{-OTA}$

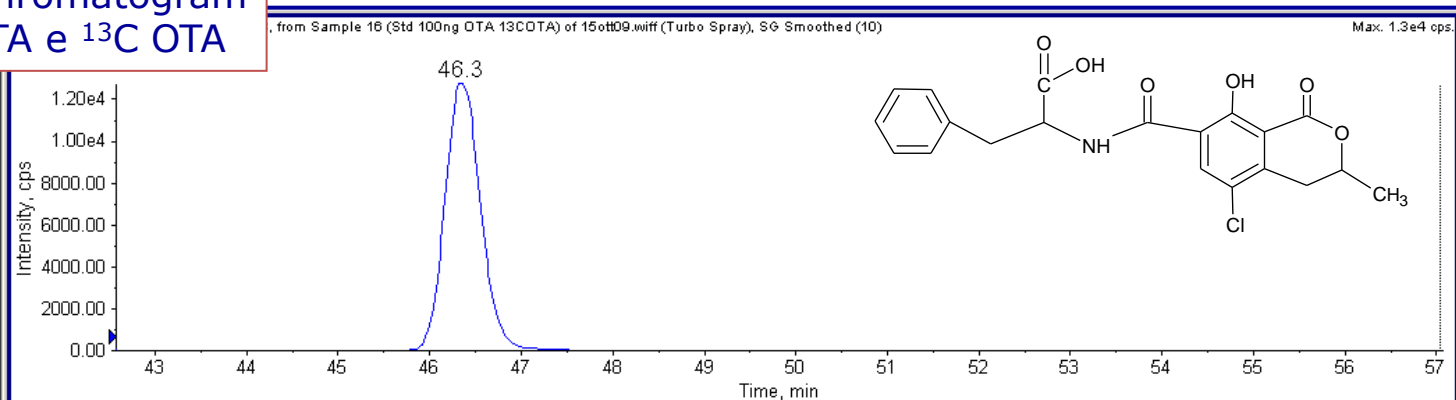
Substitution of [^{12}C] carbon atoms by [^{13}C]

Chromatographic retention time and ionization potential identical to those of naturally occurring mycotoxins.

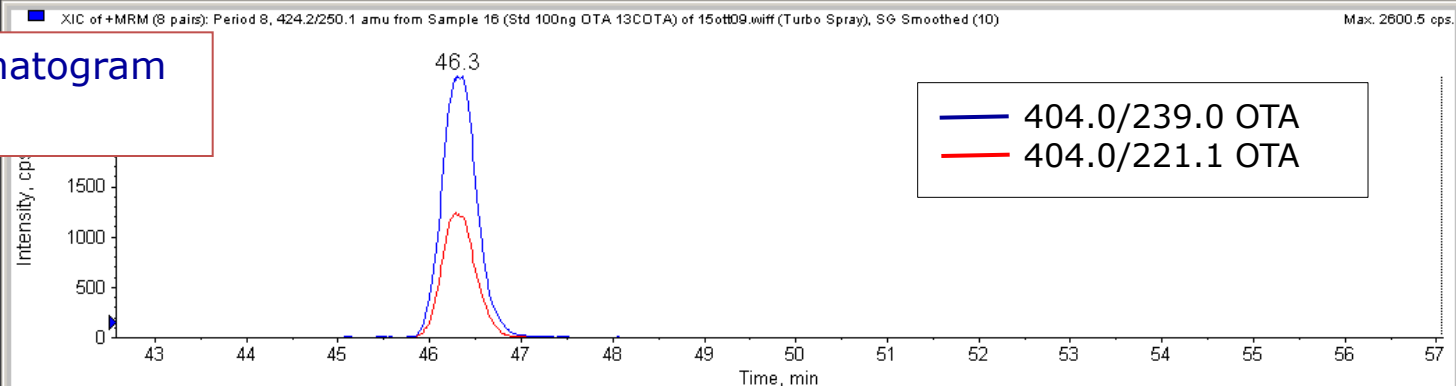
Isotope-labelled mycotoxins exhibit the **same fragmentation pattern** as their naturally occurring analogs, but because of their higher molecular weight distinction between internal standard and target analyte is possible.



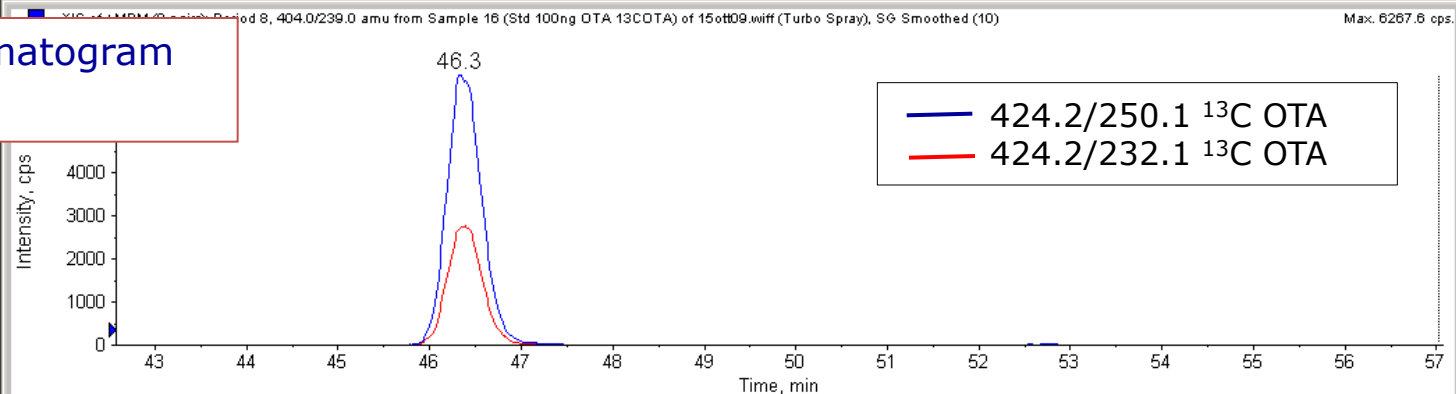
MRM Total ion chromatogram of a mixture of OTA e ¹³C OTA



Extract ion chromatogram (standard OTA)



Extract ion chromatogram (standard ¹³C OTA)



Aim of the study: to obtain information on currently used LC-MS(MS) methodologies for multi-mycotoxin analysis and relevant performances.

Participants analyzed the following materials, using their LC-MS(MS) methodology:

- ✓ one maize sample contaminated with aflatoxins (B_1 , B_2 , G_1 , G_2), OTA, DON, HT-2, ZEA, fumonisins (B_1 , B_2)
- ✓ one blank maize sample (to be spiked for recovery evaluation)

Participants determined those mycotoxins that could be simultaneously detected with their LC-MS(MS) methodology.

Solfrizzo, M., De Girolamo, A., Lattanzio, V.M.T., Visconti, A., Stroka, J., Alldrick, A. and van Egmond, H.P., 2012. Results of a proficiency test for multi-mycotoxin determination in maize by using methods based on LC-MS/(MS). Quality Assurance and Safety of Crops & Foods (*in press*)

De Girolamo A., Solfrizzo M., Lattanzio V.M.T., Stroka J., Alldrick A., van Egmond H.P., Visconti A., 2012. Critical evaluation of LC-MS-based methods for simultaneous determination of major mycotoxins (deoxynivalenol, ochratoxin A, zearalenone, aflatoxins, fumonisins, T-2 and HT-2 toxins) in maize. World Mycotoxin Journal (*submitted*)

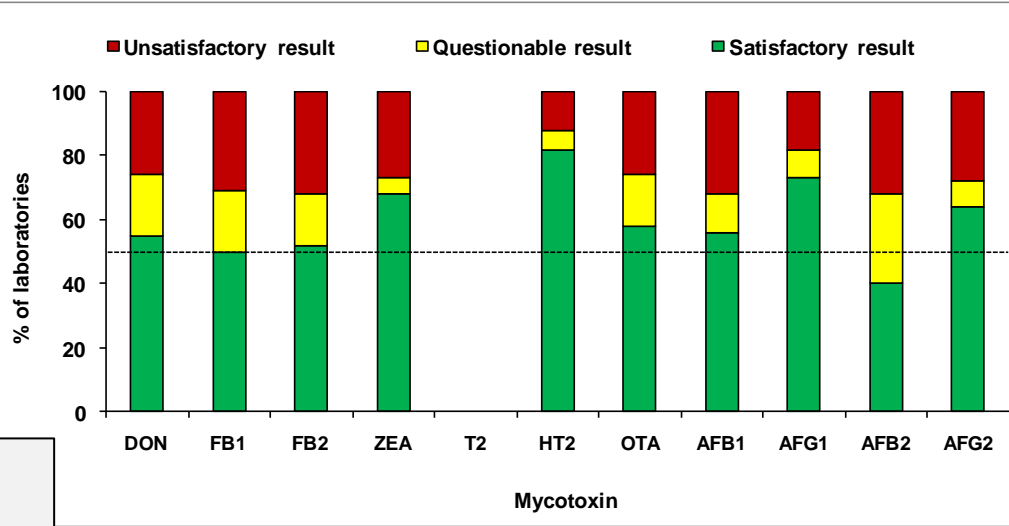
Contaminated maize

$|z| \leq 2$
 $2 < |z| \leq 3$
 $|z| > 3$

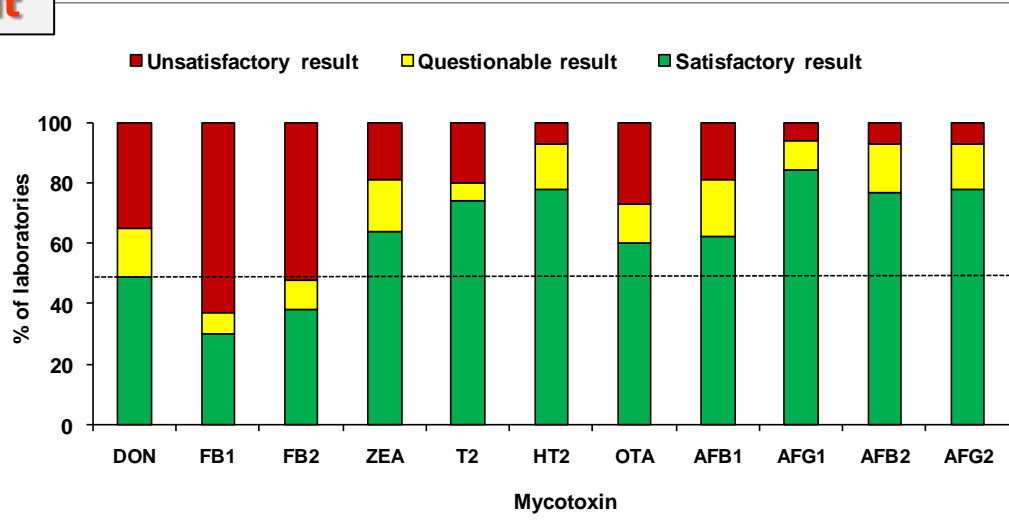
satisfactory result

questionable result

unsatisfactory result



Spiked maize



A **robust and reliable method for simultaneous determination of 11 mycotoxins in maize could not be identified** but important information on used LC-MS(MS) methodologies and relevant performances could be obtained.

❖ **Tested mycotoxins**

63% of laboratories reported results for the **11 mycotoxins**

❖ **Methodology**

54% laboratories used sample extract **cleanup**

39% laboratories analysed **crude extract**

❖ **MS conditions**

100% laboratories used **electrospray ionization**

88% laboratories used **triple quadrupole detector**

9% laboratories used **ion trap detector**

5 % laboratories used **HRMS** based on Orbitrap technology

❖ **Calibration**

44% laboratories **compensated for matrix effects**

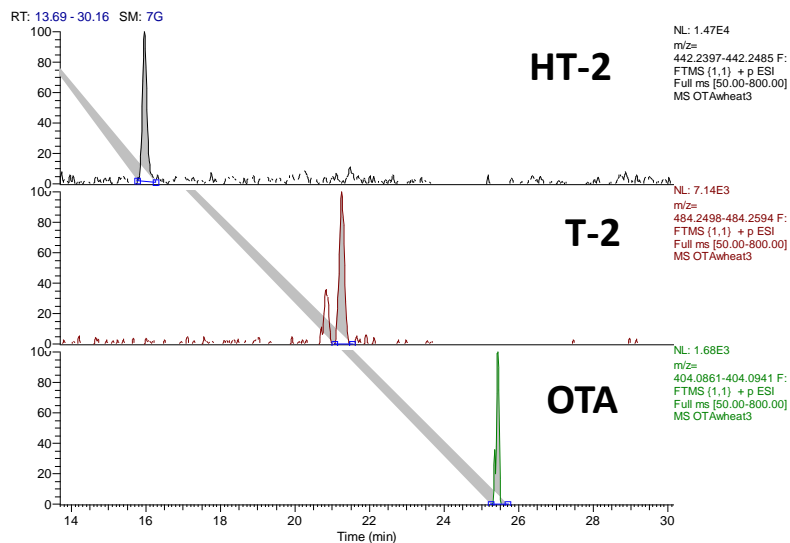
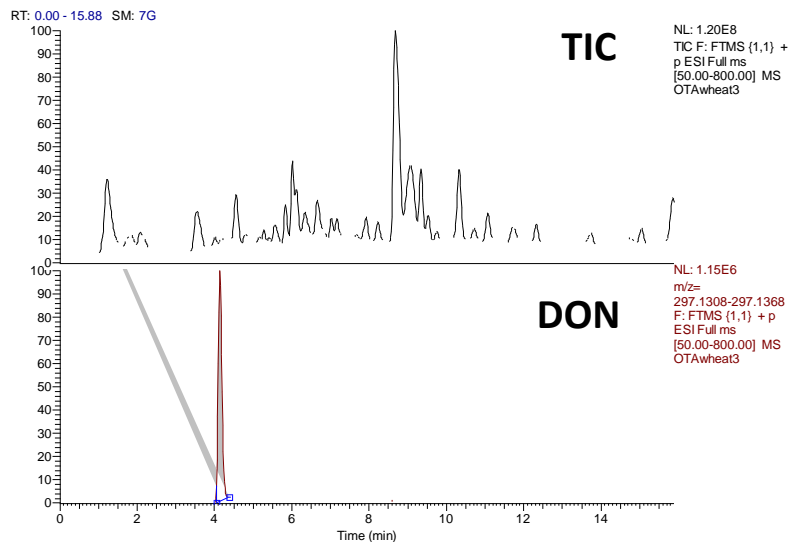
49% used **external standard calibration**

- The **direct analysis of crude extract** by LC-MS/(MS), including the dilute-and-shoot approach, gave better results than those obtained by cleaning up sample extracts.
- **Matrix-matched calibration** or **isotope internal standards** efficiently compensated for matrix effects even by injecting matrix equivalent amounts > 20 mg. *However injection of matrix amounts higher than 100 mg resulted in a decrease of sensitivity due to the increase of signal suppression.*
- **External calibration** gave reliable results when matrix equivalent amounts of ≤ 10 mg were injected.

Full Scan HRMS Metabolite discovery

The main advantage of full scan HRMS analysis is to perform **retrospective analysis**, i.e. to search for **mycotoxin metabolites/conjugates** (degradation products or “masked” forms) at a second stage without the need to re-analyze the sample.

DON derivatives in wheat flour

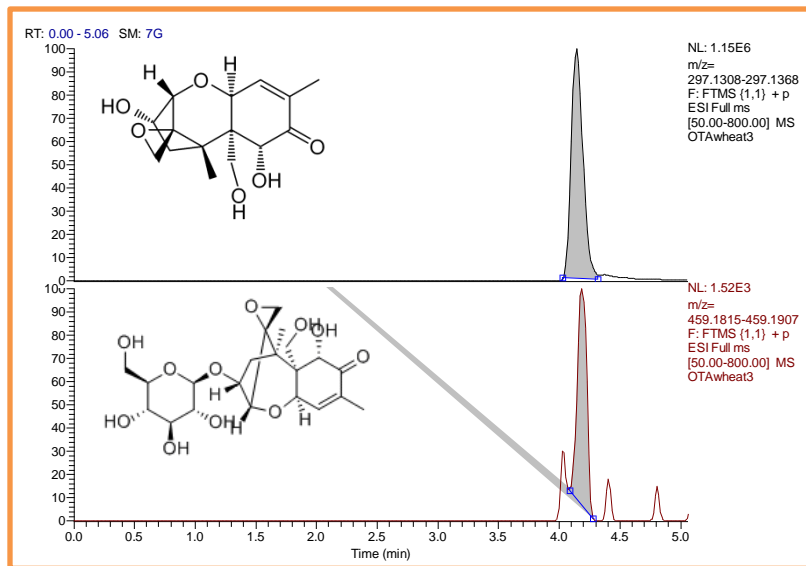


Soft wheat flour
naturally contaminated with:
898 µg/kg DON;
9 µg/kg HT-2;
2 µg/kg T-2;
6 µg/kg OTA

100.000 FWHM
Tolerance 10 ppm

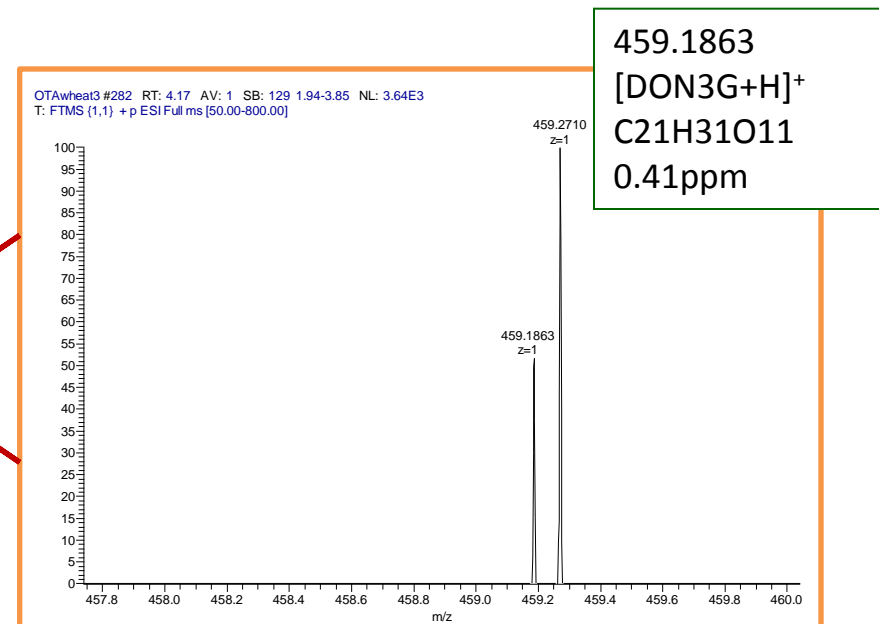
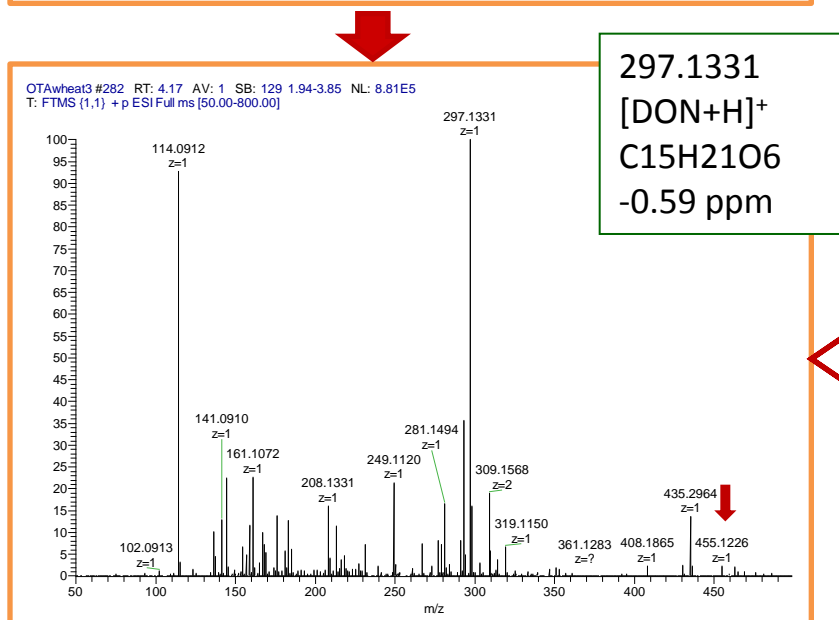
Sample preparation:
Acetonitrile/water extraction
Polymeric SPE clean up

DON derivatives in wheat flour



Deoxynivalenol-3-glucoside

1. Extract metabolite teorethical mass
2. Measured mass
3. Calculate molecular formula and mass error

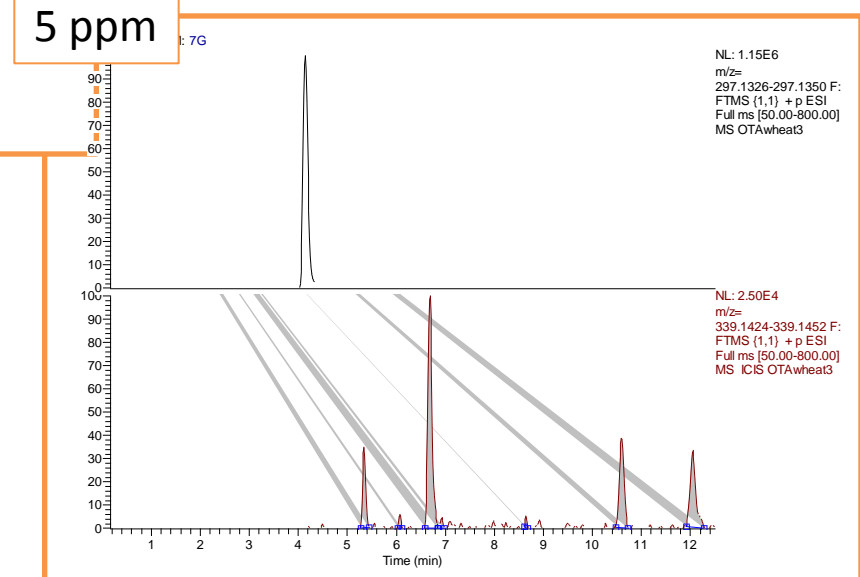
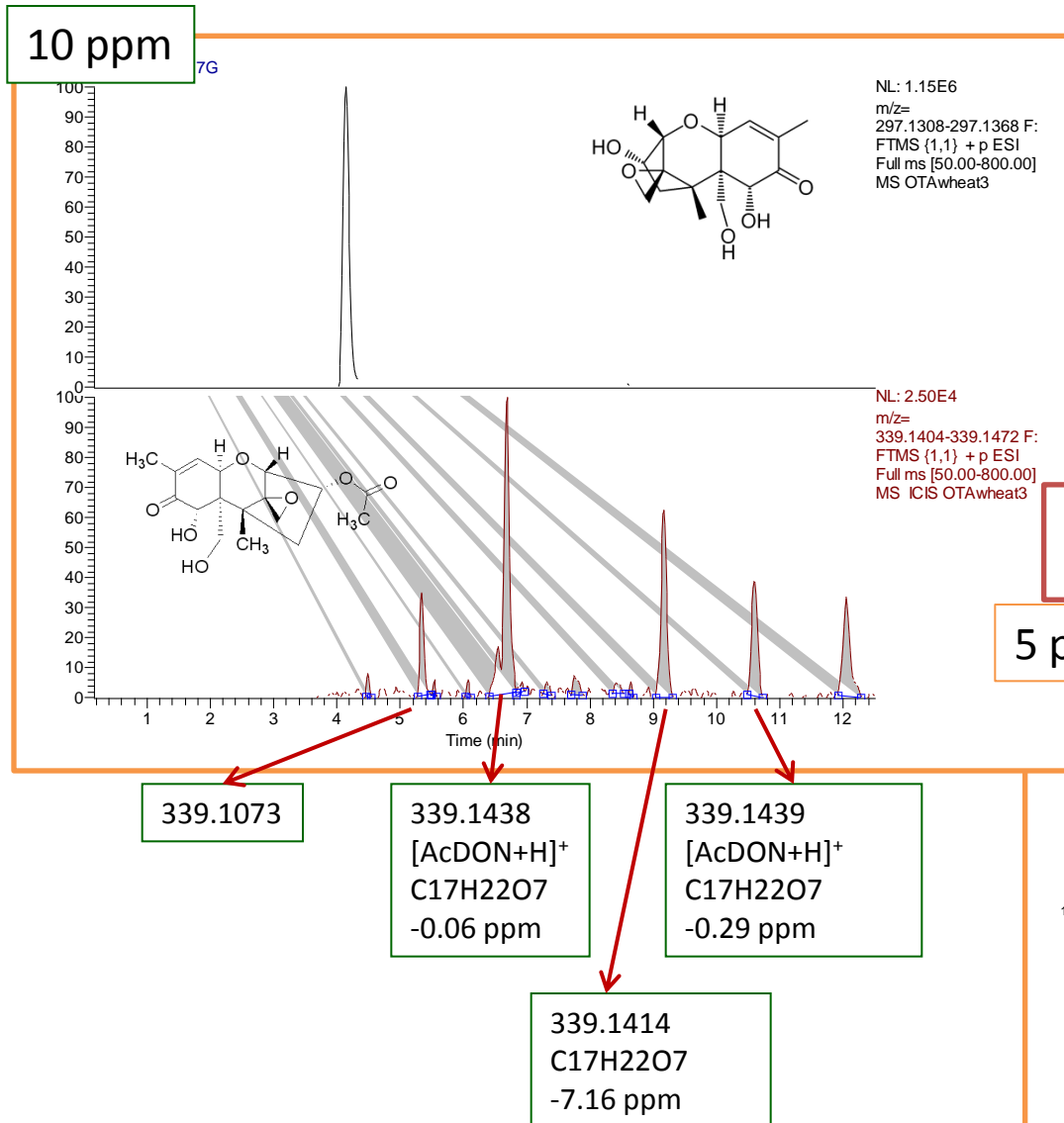


DON derivatives in wheat flour

Deoxynivalenol acetyl derivatives

1. Extract metabolite theoretical mass
2. Measured mass
3. Calculate molecular formula and mass error

Using low tolerance: unbiased compound identification

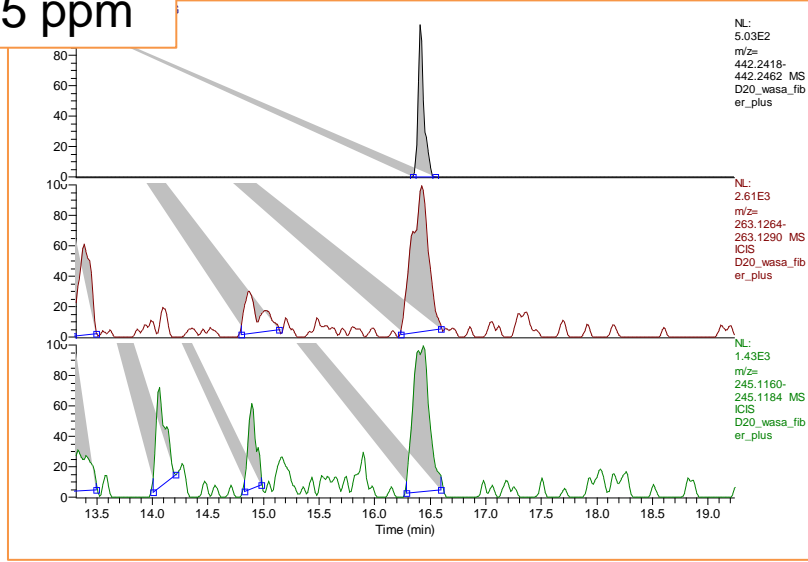




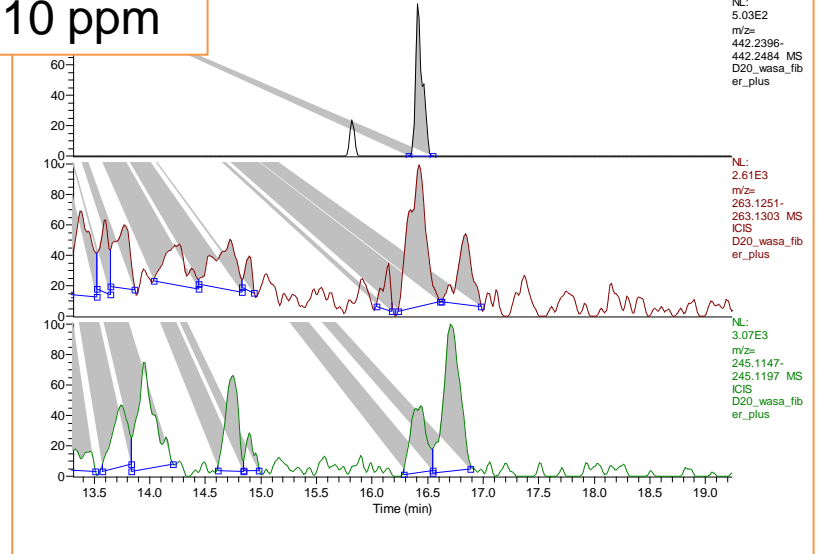
Key parameters for unbiased identification

MASS EXTRACTION WINDOW

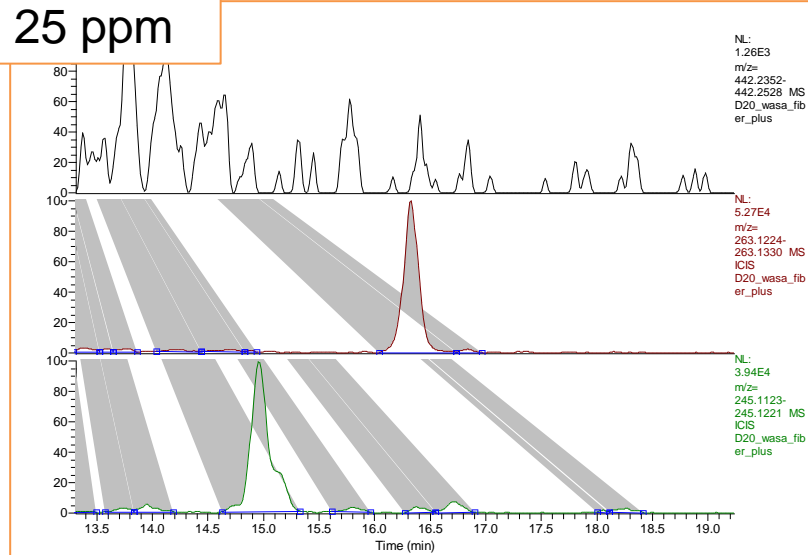
5 ppm



10 ppm



25 ppm



The **SELECTIVITY** increases with narrowing of the mass extraction window.

HT-2 (25 µg/kg) in rye based crisp bread extract

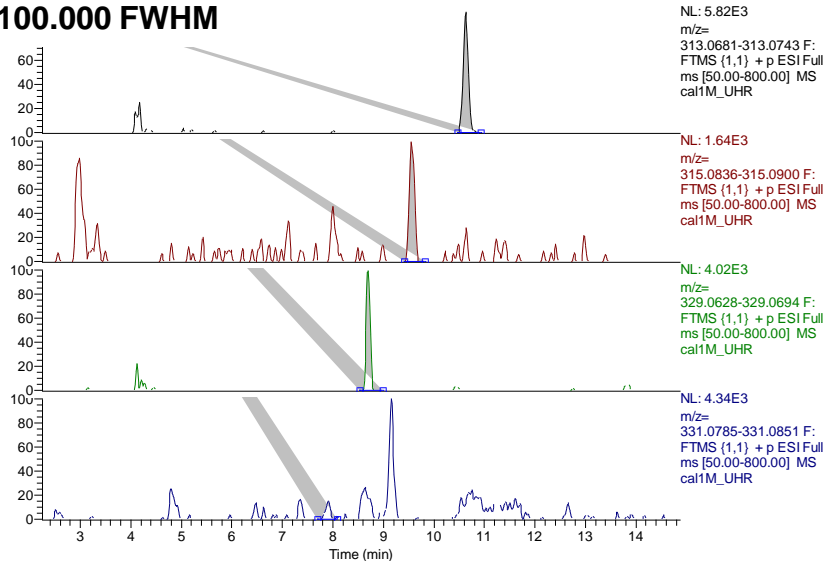
100.000 FWHM

Extracted ions:
442.2441
263.1278
245.1172

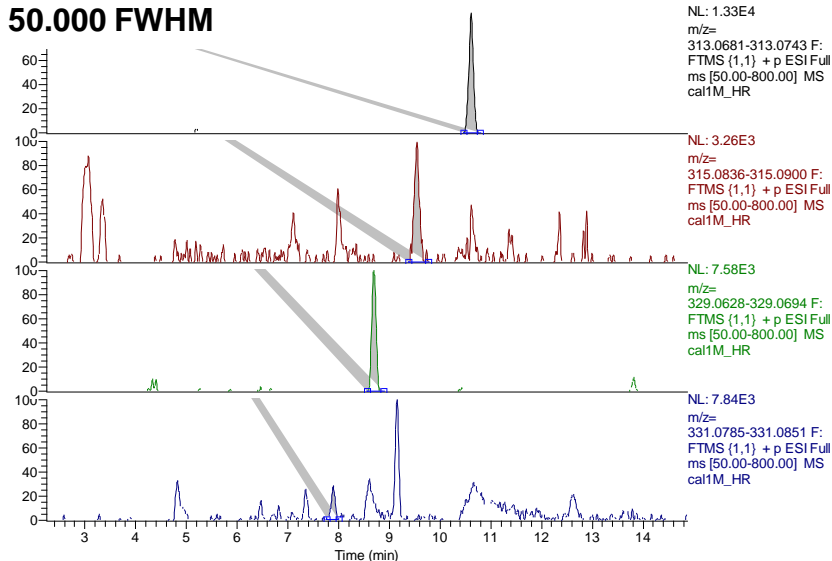


Key parameters for unbiased identification RESOLVING POWER

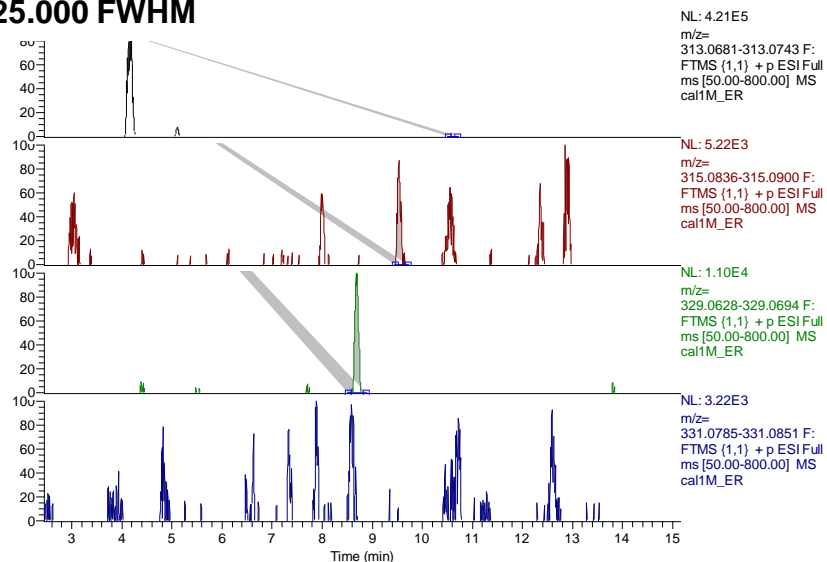
100.000 FWHM



50.000 FWHM



25.000 FWHM



Aflatoxins in barley flour extract
AFB₁ – AFG₁ 2 µg/kg
AFB₂ – AFG₂ 0.5 µg/kg

Identification and characterization of T-2 and HT-2 glycosides in wheat and oats

Oat sample naturally
contaminated with 246 µg/kg
T2 and 608 µg/kg HT2

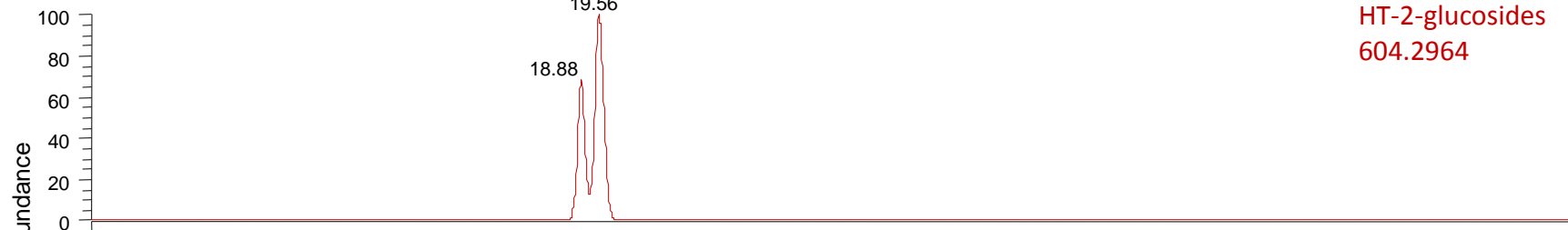
22.63

HT-2
442.2435



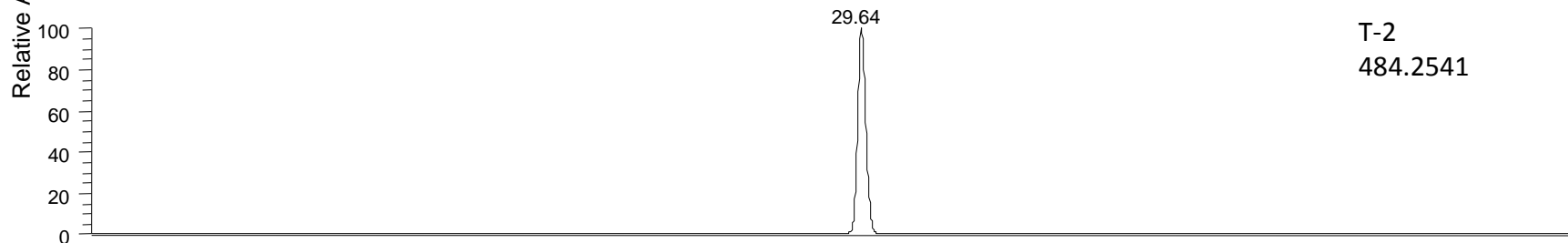
19.56
18.88

HT-2-glucosides
604.2964



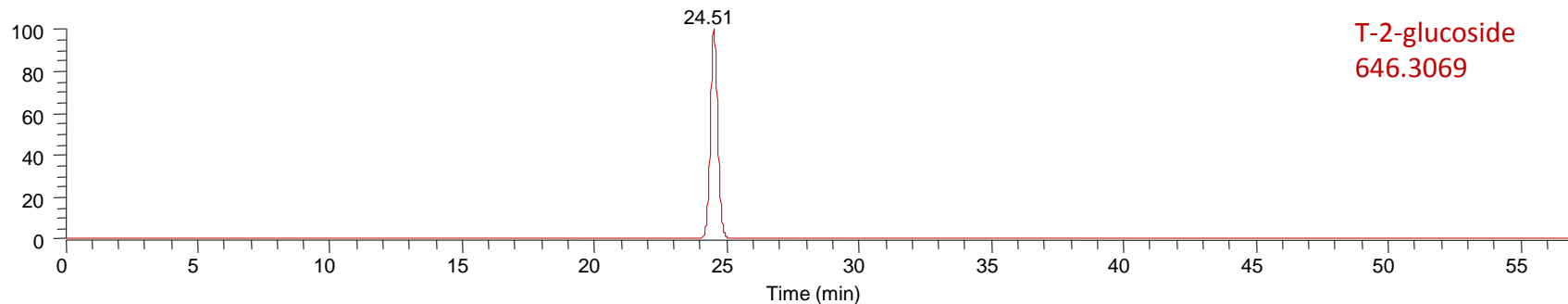
29.64

T-2
484.2541

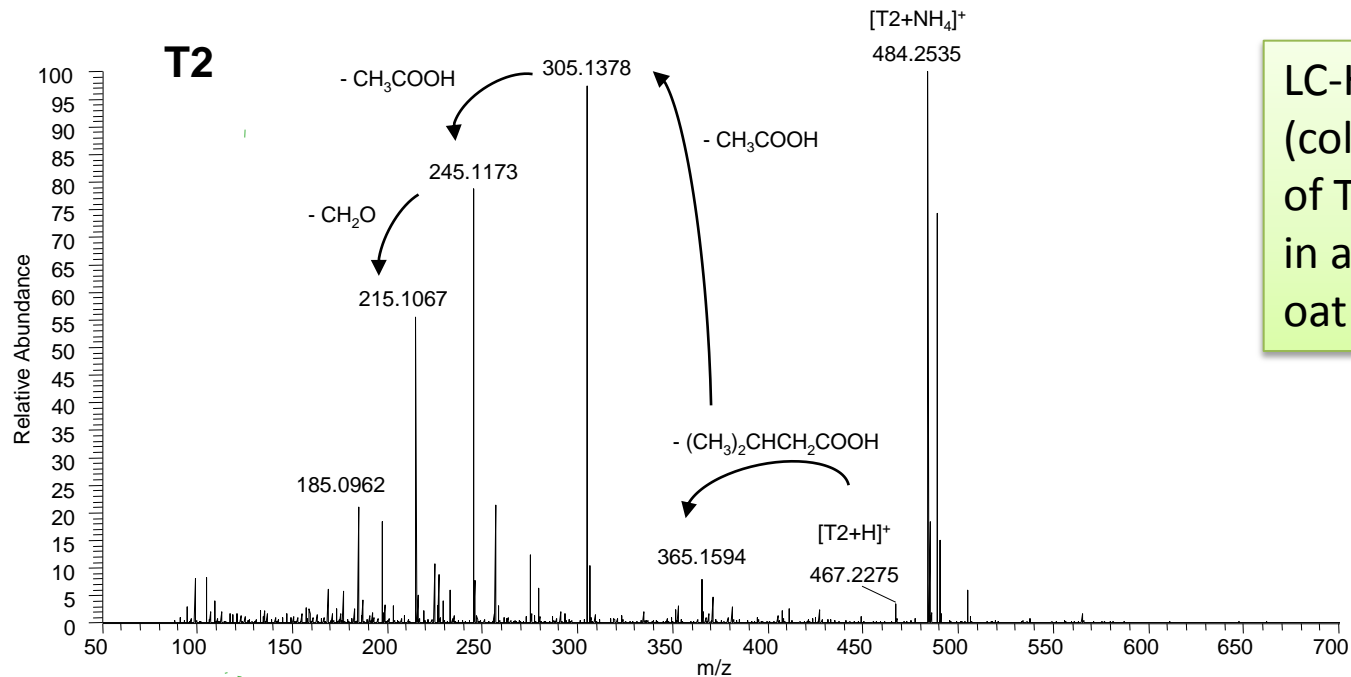


24.51

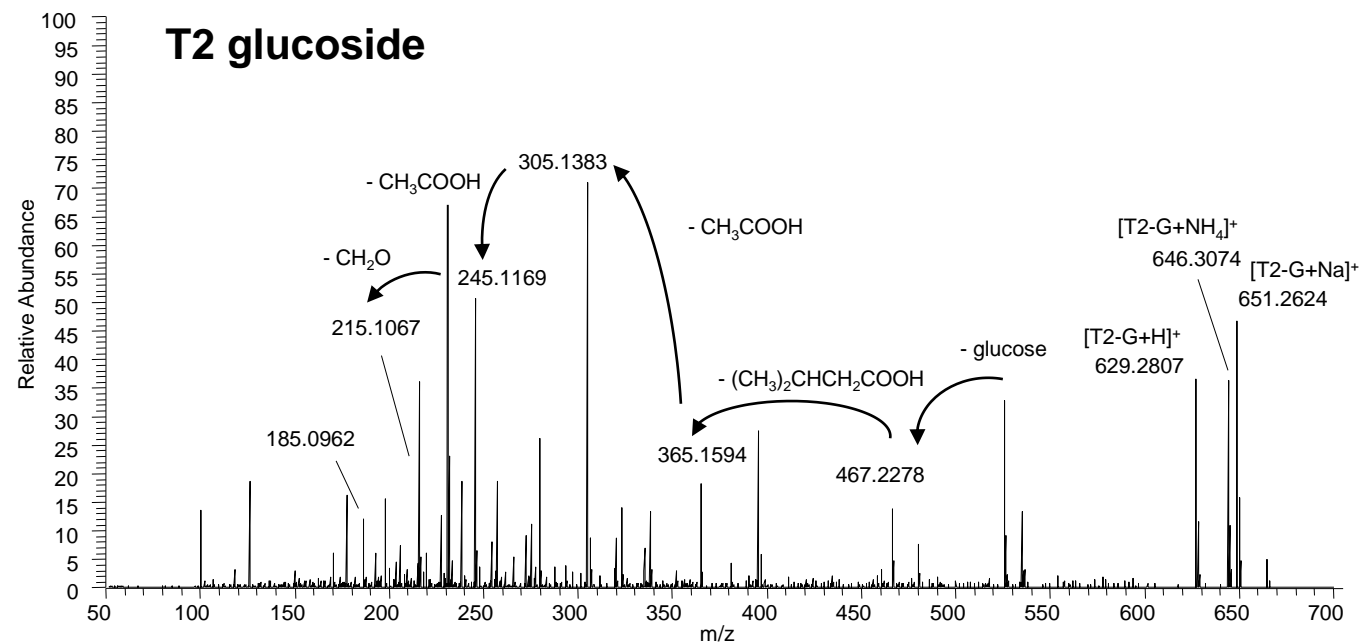
T-2-glucoside
646.3069

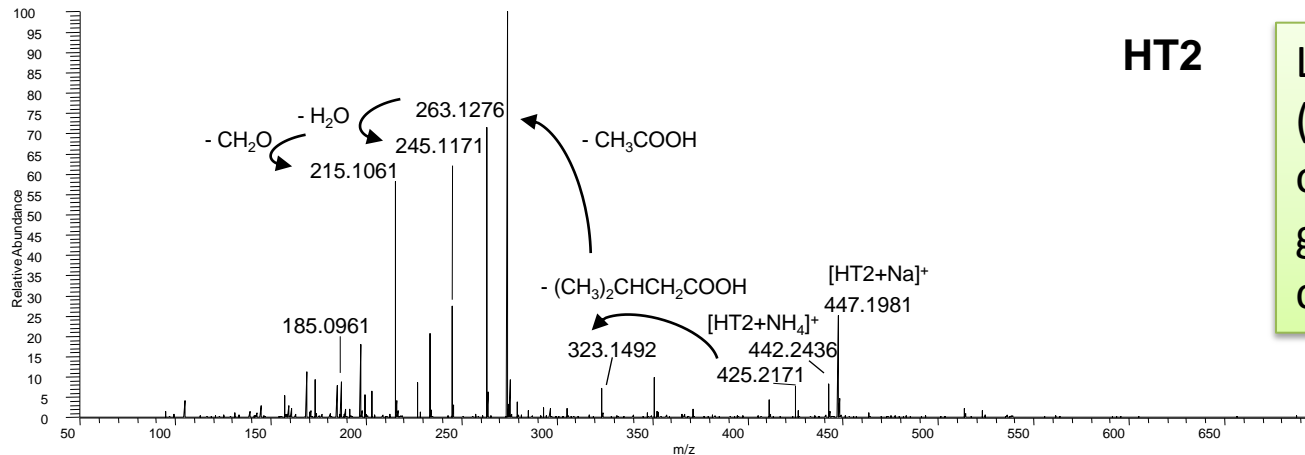




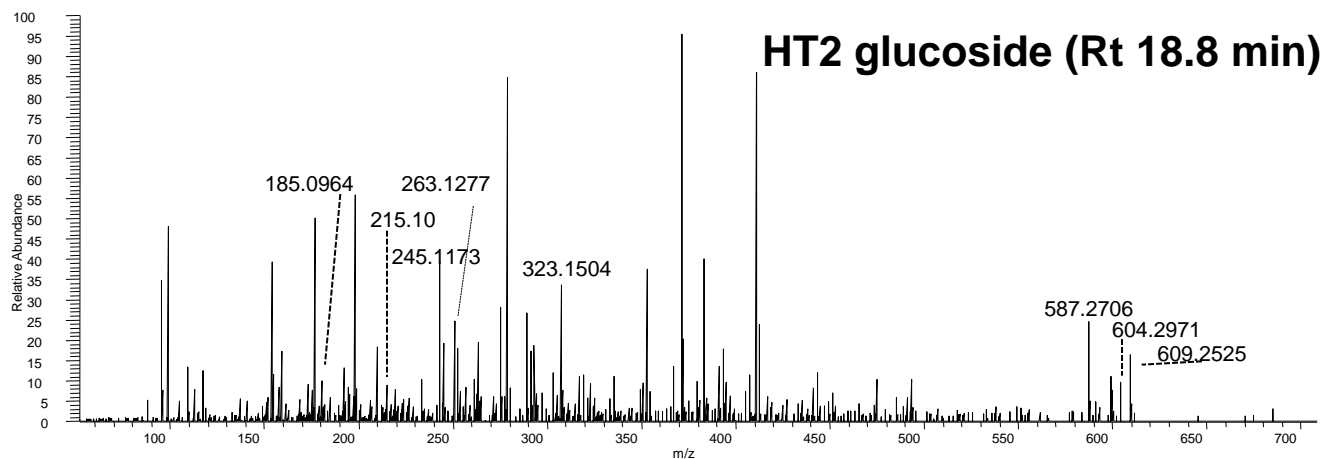
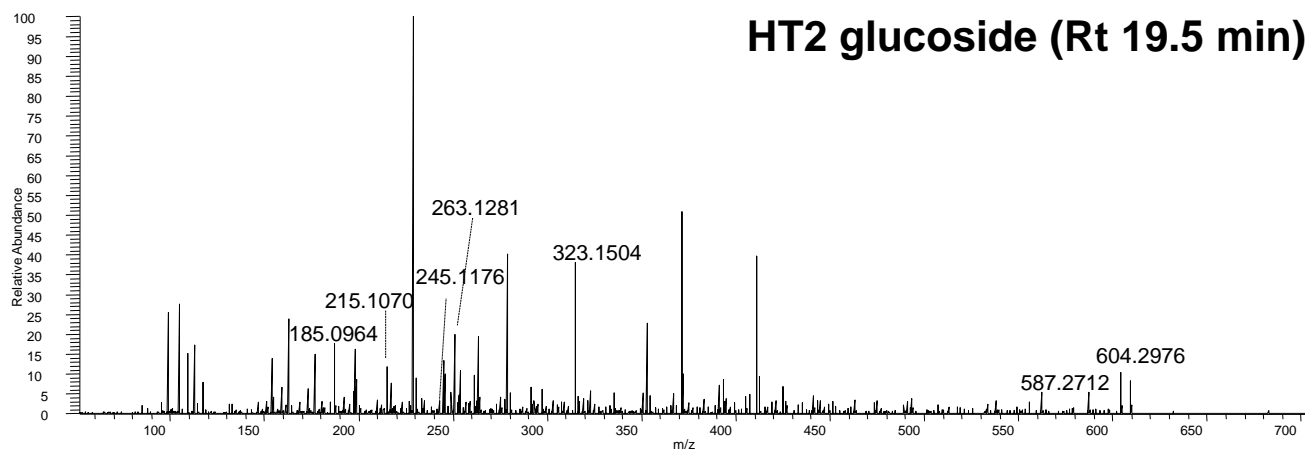
T2

LC-HCD-HRMS spectra
(collision energy 10 eV)
of T-2 toxin and T2-glucoside
in a naturally contaminated
oat sample

T2 glucoside



LC-HCD-HRMS spectra
(collision energy 10 eV)
of HT-2 toxin and HT2-
glucoside in a naturally
contaminated oat sample





CONCLUSIONS

- ✓ **Quantitative** and **confirmatory** information using HRMS (Orbitrap™ based high resolution with HCD fragmentation) or MS/MS (low resolution – triple quadrupole) can be obtained, meeting European regulatory requirements for confirmatory analysis.
- ✓ Analytical procedures based on either LC-MS/MS or LC-HRMS can exhibit satisfactory recoveries and adequate detection limits to assess mycotoxin contamination in cereals and cereal foods at regulatory levels.
- ✓ **Matrix assisted calibration** is an essential requirement for proper analyte quantification.
- ✓ The main advantage of full scan HRMS analysis is to perform **retrospective analysis**, i.e. to search for **mycotoxin metabolites/conjugates** (degradation products or “masked” forms) at a second stage without the need to re-analyze the sample.

THANK
YOU!

