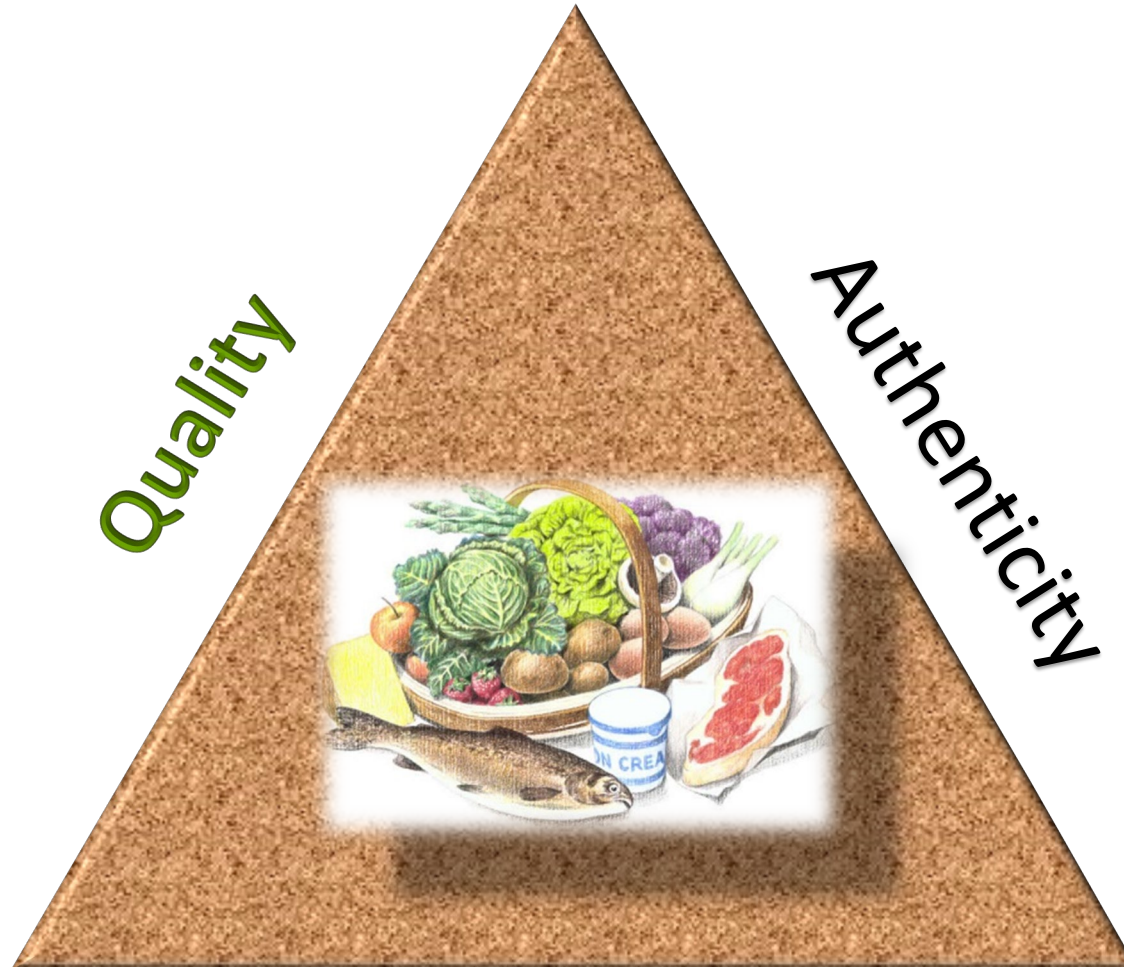


Possibility to integrate analysis of pesticide residues, mycotoxins and plant alkaloids in a single multi-analyte / multi-matrix method

Jana Hajšlová, Zbyněk Džuman, Vladimír Kocourek

KEY RESEARCH FOCUS: FOOD INTEGRITY



SAFETY

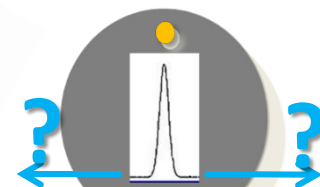
'COCKTAILS' of food contaminants (?)

Commodity	meat	cereals	nuts & seed	fats & oils	fish	milk & eggs	tea & coffee	vegetable	fruit	herbs	honey
HAZARD											
natural toxins		+			+++	+	+	+			++
mycotoxins		+++	+++			++	+	+	++	+++	
industrial pollutants	++	+		+	+++	++	+	++	+	+	+
toxic metals	+	+			++		+	++	+	+	
pesticide residues	+	++	++	+		++	+	+++	+++	+++	++
veterinary drug residues	++	+	+		+	+++	+			+	+
migrants from plastics	+			+		++	+	+	+	+	+
process contaminant		++		++	+		++				

Complementary analytical strategies

'CLASSIC' APPROACH

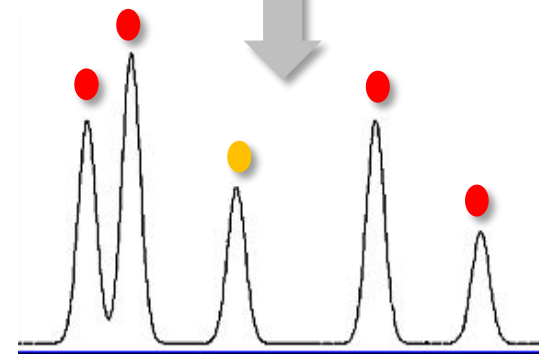
A set of physico-chemical and/or biochemical measurements → **TARGET ANALYSIS** of one or few quality / safety markers



CURRENT STRATEGY

Metabolomic fingerprinting

→ **NON TARGET SCREENING**



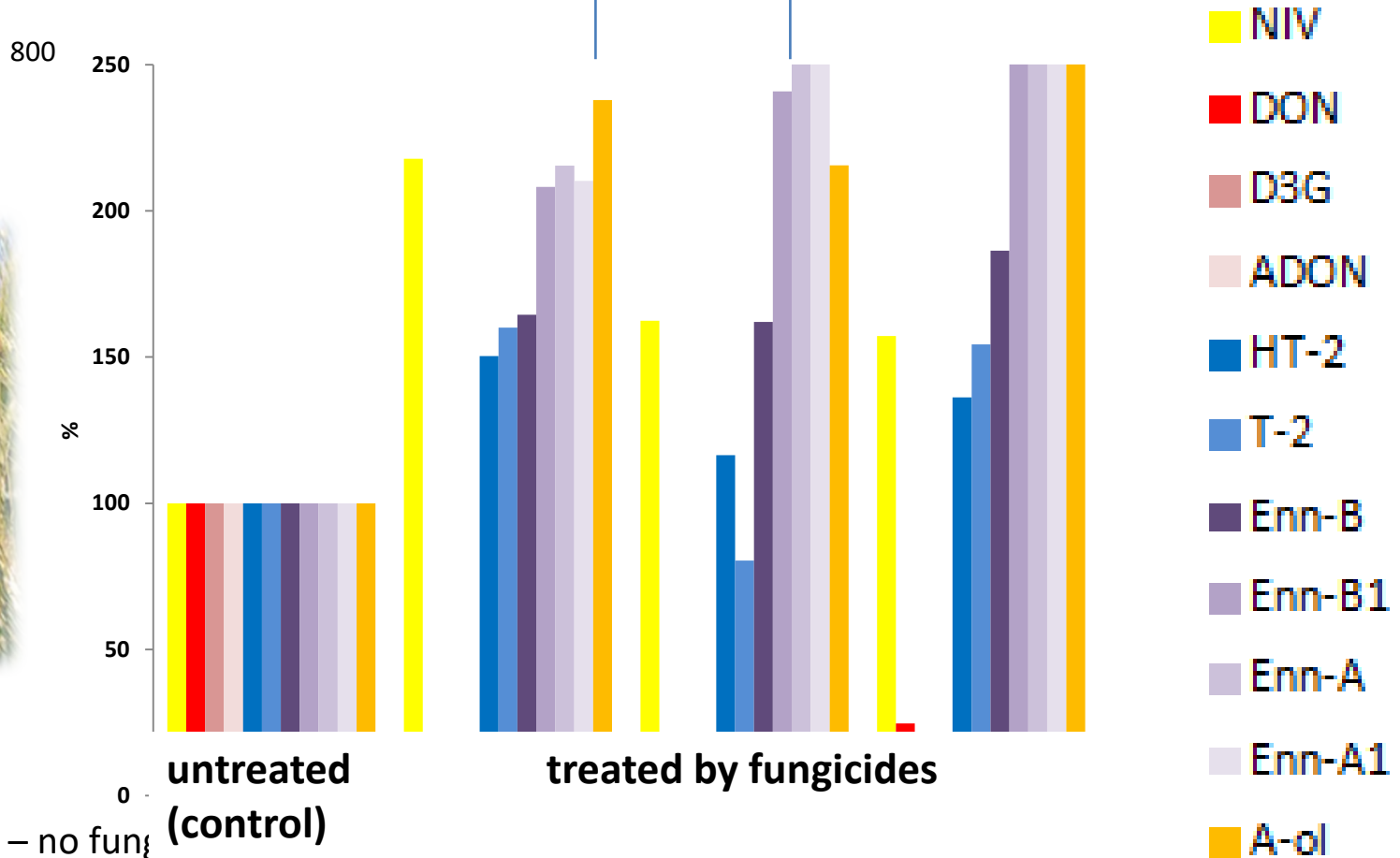
- ➔ Comprehensive characterization of respective matrix (sample components pattern)
- ➔ detection or even identification of 'unknown' metabolites / contaminants (even retrospective)

Changing food safety issues with changing climate



Unexpected problems might be faced....

eliminating DON producers results in high overgrowing of producers of enniatins, T-2/HT-2, NIV and other in barley



untreated (1) – no fungicide

treated (2) - prothioconazol, spiroxamin a tebuconazol + tebuconazol a bixafen

treated (3) - prothioconazol, spiroxamin a tebuconazol + prothioconazol a tebuconazol

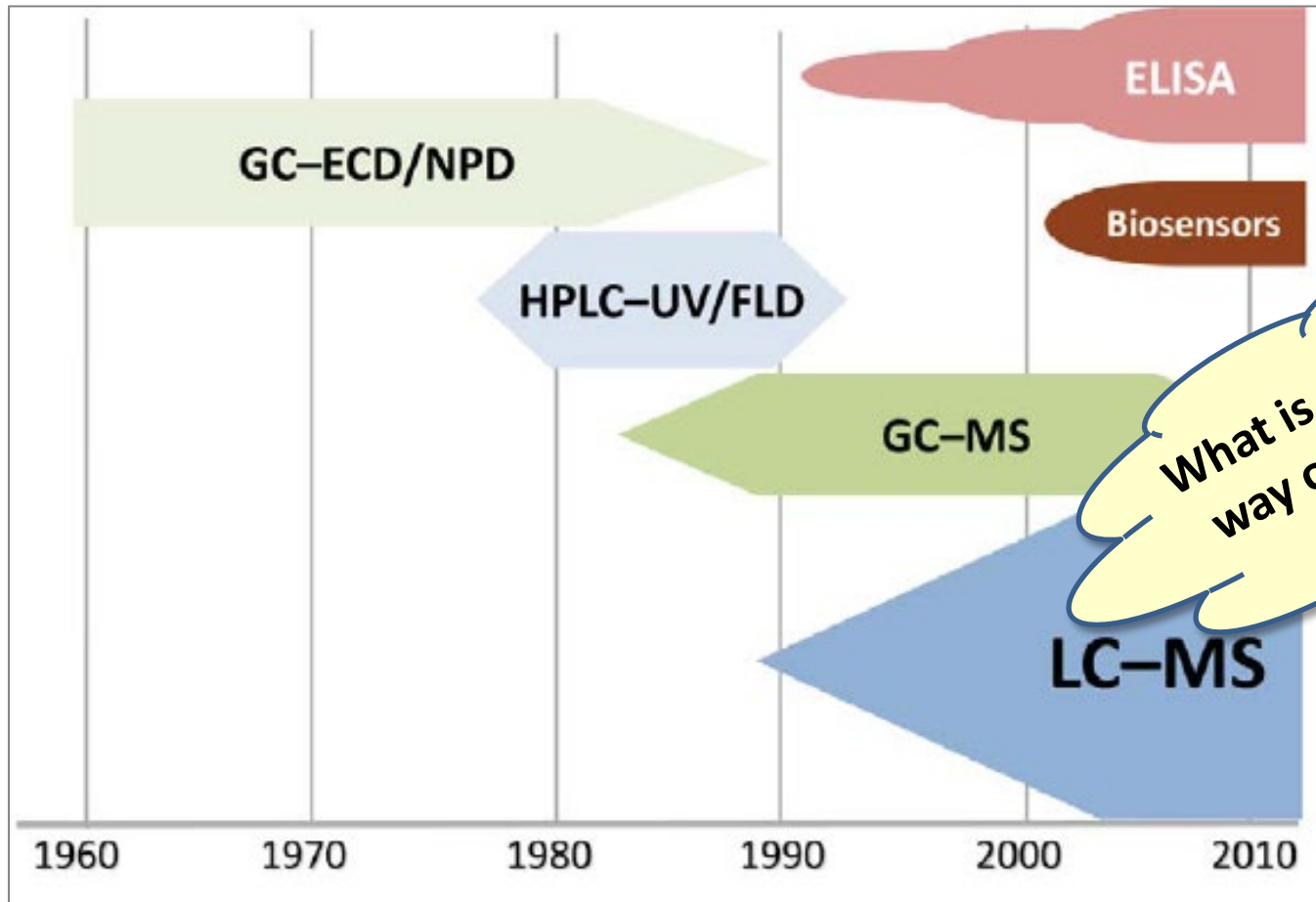
treated (4) - variant (2) with differing application dates as compared to (3)

MULTIPLE GROUPS OF CONTAMINANTS MAY OCCUR IN FOOD / FEEDS.....

→ implementation of multi-analyte / multi-matrix methods may increase the laboratory throughput and lower the analysis cost



TIME TRENDS IN FOOD CONTAMINANTS ANALYSIS



What is the most effective way of these techniques exploitation ?



Multiple contaminants analysis (sample prep)



Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma



Critical assessment of extraction methods for the simultaneous determination of pesticide residues and mycotoxins in fruits, cereals, spices and oil seeds employing ultra-high performance liquid chromatography–tandem mass spectrometry

Ondrej Lacina, Milena Zachariasova, Jana Urbanova, Marta Vaclavikova, Tomas Cajka, Jana Hajslova*

METHOD

- A – aqueous acetonitrile extraction followed by partition (QuEChERS-like method)**
- B – aqueous acetonitrile extraction**
- C – *pure acetonitrile extraction (?)***

ANALYTES

- 288 pesticides (including ‘troublesome’ acidic, basic and base-sensitive compounds)**
- 38 mycotoxins (including all EU regulated ones and many ‘emerging’ toxins on the EFSA list)**

MATRICES

- apple baby food, wheat flour, spices and sunflower seeds,**

Multiple contaminants analysis (HRMS/MS)



Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca



Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids



Zbynek Dzuman^a, Milena Zachariasova^{a,*}, Zdenka Veprikova^a, Michal Godula^b,
Jana Hajslova^a
ACA 863 (2015) 29–40

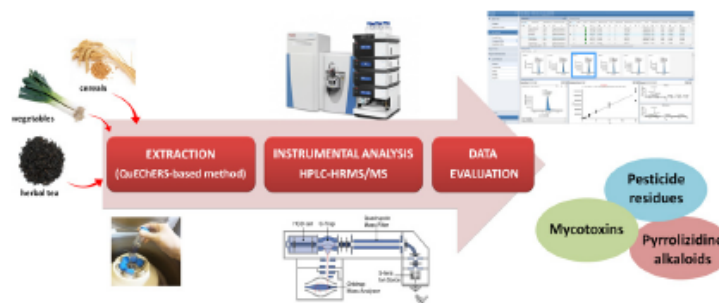
^a University of Chemistry and Technology, Prague, Technicka 3, Prague 6, 16628, Czech Republic

^b Thermo Scientific, Slunecna 27, Prague 10, 10000, Czech Republic

HIGHLIGHTS

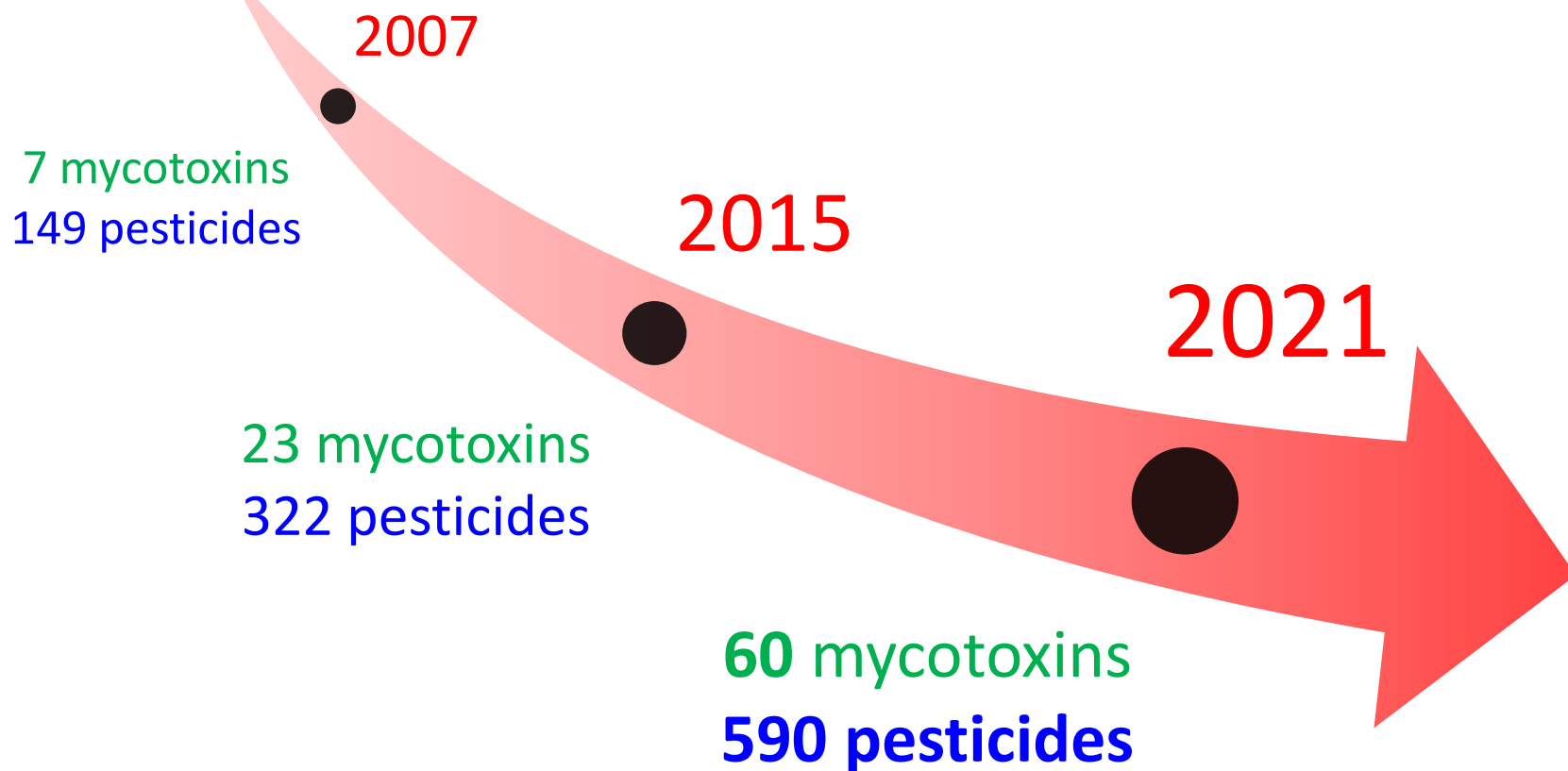
- HPLC–HRMS/MS method for analysis of 389 multi-class food contaminants was developed.
- The employed core–shell analytical column showed very good separation efficiency.
- Validation for matrices wheat, leek, and tea was performed.
- Recoveries and limits of quantification complied with the EU legislation.
- The mass spectral library of fragment ions in high resolution was created.

GRAPHICAL ABSTRACT



■ Mycotoxins and pesticides we have in the scope (LC-HRMS/MS accredited method)

- Determination of **mycotoxins** and **pesticide residues** at UCT using liquid chromatography coupled with mass spectrometry.



Analytical strategy

- Currently, the most frequently used techniques are LC–MS (mycotoxins) and LC/GC–MS (pesticide residues)
 - Detection typically realized using mass spectrometers equipped with unit resolution analyzers
- Potential of **high resolution tandem mass spectrometry** exploited
 - **Analysis of complex samples** (spices, tea, dietary supplements, etc.)
 - **Retrospective data analysis**
 - **Targeted screening**
- ✗ *Limitation: acquisition of voluminous data, data processing (powerful PC), data storage*



Analytical flow-chart

Sample

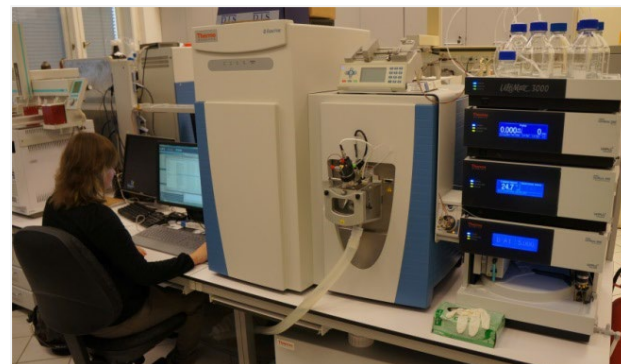
➤ 1 g of dry sample

Extraction (QuEChERS-like method)

- (i) 30 min soaking (10 mL 0.2% formic acid in water) + 30 min extraction (10 mL MeCN)
- (ii) addition of 4 g MgSO_4 and 1 g NaCl; 2 min shaking; centrifugation (10,000 RPM, 5 min)
- (iii) clean-up: 5 mL of organic phase for 2h at -20°C (freezer)
- (iv) transfer of 1 mL into an amber vial

U-HPLC-HRMS/MS screening

Separate methods in ESI+/- using different mobile phases
ESI+: 5 mM ammonium formate + MeOH (0.2% HCOOH)
ESI-: 5 mM ammonium acetate + MeOH



◀ Quantification strategy:
**external matrix-matched
calibration**

PERFORMANCE
CHARACTERISTICS ▶

Analyte group	LOQ [$\mu\text{g}/\text{kg}$]	Recovery [%]
Pesticides	1 – 1,000	59 – 116
Mycotoxins	1 – 2,000	71 – 111
Pyrrolizidine alkaloids	1 – 20	72 – 94



Data processing

Thermo
SCIENTIFIC

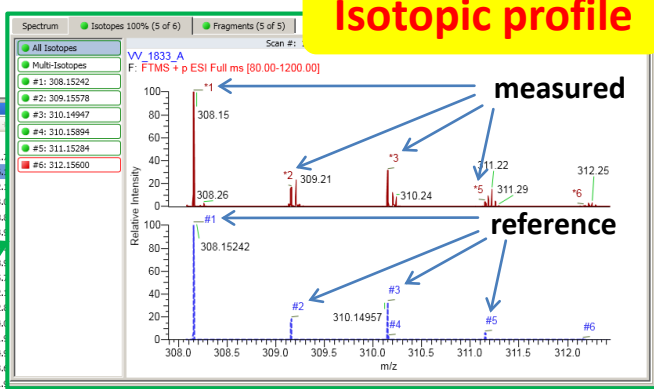
SAMPLES

Sample Name	Color
VV_1833_A	Green
VV_1833_B	Green
VV_1833_C	Green
VV_1834_A	Green
VV_1834_B	Green
VV_1834_C	Green
VV_1844_A	Green
VV_1844_B	Green
VV_1844_C	Green
VV_1869_A	Red
VV_1869_B	Red
VV_1869_C	Red
VV_1871_A	Green
VV_1871_B	Green
VV_1871_C	Green
mstd_100_a	Green

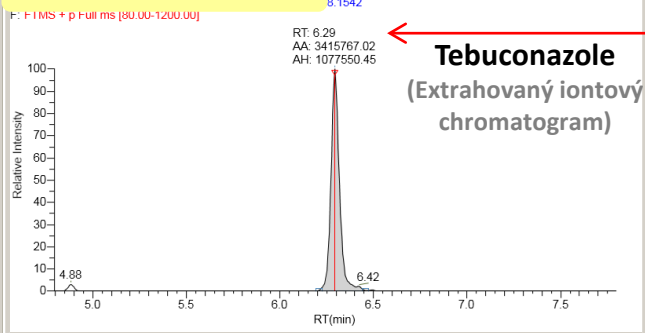
DETECTED ANALYTES

RT (min)	MZ	IP	FI	LS	Compound	Formula	Charge	Confirms	m/z (Expected)	m/z (Apex)	m/z (Delta (ppm))	RT (Expected)
6.29	308.15	✓	✓	✓	tebuconazole	C16H22ClN3O	M+H+	3 out of 3	308.1524	308.1518	-1.9652	6.29
2.38	267.15	✓	✓	✓	verrucarol	C15H22O4	M+H+	3 out of 3	267.1591	267.1588	-0.9583	2.38
3.12	222.11	✓	✓	✓	carbofuran	C12H18NO3	M+H+	2 out of 3	222.1125	222.1122	-1.4976	3.12
4.32	326.37	✓	✓	✓	DDAC (didecyldimethylammonium chloride)	C29H48N	M+H+	2 out of 3	326.3781	326.3776	-1.5488	4.32
5.82	192.13	✓	✓	✓	DEET	C12H17NO	M+H+	2 out of 3	192.1383	192.1382	-0.6058	5.82
8.95	268.15	✓	✓	✓	diethofencarb	C14H21NO4	M+H+	2 out of 3	268.1540	268.1539	-1.4911	8.95
5.82	275.20	✓	✓	✓	empenthrin	C18H26O2	M+H+	2 out of 3	275.2006	275.1998	-2.6559	5.82
5.82	337.12	✓	✓	✓	fenbuconazole	C19H17ClN4	M+H+	2 out of 3	337.1215	337.1208	-2.2000	5.82
2.81	262.11	✓	✓	✓	imazapyr	C13H15N3O3	M+H+	2 out of 3	262.1186	262.1181	-1.8956	2.81
2.81	166.08	✓	✓	✓	metholcarb	C9H11NO2	M+H+	2 out of 3	166.0863	166.0863	-0.0695	2.81
3.97	285.12	✓	✓	✓	metominostrobin	C16H16N2O3	M+H+	2 out of 3	285.1234	285.1230	-3.2721	3.97
2.37	166.08	✓	✓	✓	retorsine N-oxide	C18H25NO7	M+H+	2 out of 3	166.0863	166.0863	-0.0695	2.37
4.88	298.27	✓	✓	✓	spiroxamine	C18H35NO2	M+H+	2 out of 3	298.2704	298.2737	-1.4026	4.88
3.58	198.11	✓	✓	✓	TeA	C10H15NO3	M+H+	2 out of 3	198.1125	198.1129	2.4264	3.58
2.02	292.02	✓	✓	✓	thiamethoxam	C8H10ClN3O3	M+H+	2 out of 3	292.0266	292.0258	-2.5738	2.02
2.2	220.09	✓	✓	✓	2-NOA	C12H10O3	M+NH4+	1 out of 3	220.0968	220.0965	-1.3786	2.2
1.94	255.13	✓	✓	✓	3-hydroxy-carbifuran	C12H15NO4	M+NH4+	1 out of 3	255.1339	255.133	-3.485	2.22

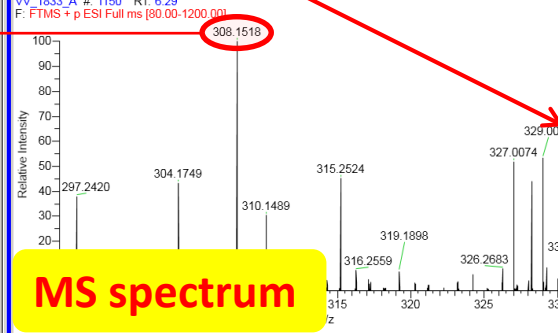
Isotopic profile



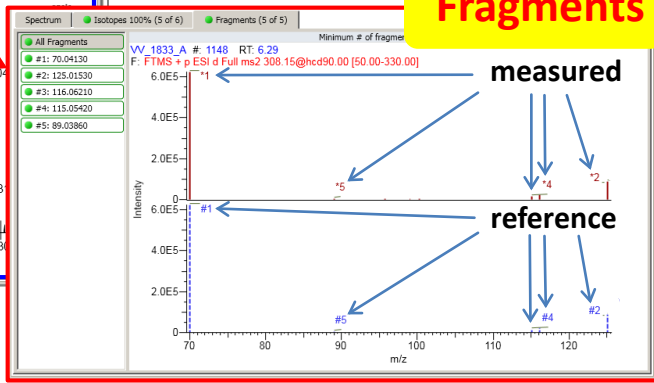
CHROMATOGRAM



MS spectrum



Fragments



FOOD SUPPLEMENTS

And what about
contamination
issue....



Samples involved in the validation study



NO.	SAMPLE	PRODUCER	SAMPLE TYPE	PACKING
1	Herbal tea for immune support	Fytopharma	tea blend	20 x 1.5g
2	Tea blend for liver support	MEGAFYT PHARMA	tea blend	20 x 1.5g
3	St. John's wort	LEROS	tea blend	20 x 1.5g
4	Renal tea blend with cranberries	MEGAFYT PHARMA	tea blend	20 x 1.5g
5	Hepatic herbal tea	Valdemar Grešik - NATURA	tea blend	50 g
6	MEGAFYT te from elderberry flower	MEGAFYT PHARMA	tea blend	20 x 1.5g
7	MEGAFYT children's tea blend	MEGAFYT PHARMA	tea blend	20 x 1.5g
8	MEGAFYT urologic tea blend	MEGAFYT PHARMA	tea blend	20 x 1.5g
9	MEGAFYT gall tea blend	MEGAFYT PHARMA	tea blend	20 x 1.5g
10	MEGAFYT stomach tea blend	MEGAFYT PHARMA	tea blend	20 x 1.5g
11	MEGAFYT sage leaves	MEGAFYT PHARMA	tea blend	20 x 1.5g
12	MEGAFYT agrimony	MEGAFYT PHARMA	tea blend	20 x 1.5g
13	LEROS ALVISAN NE	LEROS	tea blend	20 x 1.5g
14	LEROS REDU	LEROS	tea blend	20 x 1.5g
15	LEROS E	LEROS	tea blend	20 x 1.5g
16	LEROS	LEROS	tea blend	20 x 1.5g
17	LEROS S	LEROS	tea blend	20 x 1.5g
18	LEROS SPE	LEROS	tea blend	20 x 1.5g
19	LEROS THÉ SA	LEROS	tea blend	20 x 1.5g
20	LEROS STOMARAN ind	LEROS	tea blend	20 x 1.5g
21	LEROS URCYSTON urinary tract dis	LEROS	tea blend	20 x 1.5g
22	LEROS SPECIES NERVINAE for sleep	LEROS	tea blend	20 x 1.5g
23	LEROS TORMENTAN diarrhea	LEROS	tea blend	20 x 1.5g

NO.	SAMPLE	PRODUCER	SAMPLE TYPE	PACKING
43	Echinaceové drops galmed	Biomedica	herbal drops	50 ml
44	TOPVET purple coneflower	Dr. Jiří Pantůček	herbal drops	100 ml
45	Imunit Echinacea drops	Simply You Pharmaceuticals	herbal drops	50 ml + 10 ml
46	Valerian drops	MVDR. Jiří Pantůček	herbal drops	50 ml
47	Herbal drops GINKGO biloba	Hana Tajdušová	herbal drops	35 ml
48	Tropaeolum majus	Herba Vitalis	herbal drops	40 ml
49	Kräutertropfen Forte	Dr. Theiss Naturwaren GmbH	herbal drops	50 ml
50		Valdemar Grešik - NATURA	herbal drops	50 ml
51		Valdemar Grešik - NATURA	herbal drops	50 ml
52		Calendula	herbal drops	25 ml
53		Underberg GmbH & Co.KG	herbal drops	20 ml
54		Herba Vitalis	herbal drops	40 ml
55		Petr Gasparik	herbal drops	50 ml
56	Dr.Theiss Schweden Bitter	Dr. Theiss Naturwaren GmbH	herbal drops	250 ml
57	Strogast	STEIGERWALD	herbal drops	20 ml
58		IVA	herbal drops	50 ml
59		Czech Industries	herbal drops	25 ml
60		ca SE	herbal drops	50 ml
61		ca SE	herbal drops	50 ml
62		Klosterfrau	herbal drops	95 ml
63			herbal drops	100 ml
64			herbal drops	100 ml
65	Mucoplant		herbal drops	100 ml + 50 ml
66	Silvanbio		herbal drops	100 ml
67			herbal drops	100 ml
68			herbal drops	100 ml
69	STOPKASEL drops		herbal drops	100 ml + 50 ml
70	Plantain drops	FYTONA	herbal drops	150 g
71	VĚTRANKA herbal drops	T-STRING Pardubice, DIAVITA	herbal drops	108 g
72		PURUS-MEDA	herbal drops	25 ml
73		BIOMEDICA	herbal drops	100 ml
74		SANDOZ, Lek Pharmaceuticals d.d.	herbal drops	50 ml
75		Krewel Meuselbach GmbH	herbal drops	20 ml
76		Dr. Willmar Schwabe	herbal drops	20 ml
77		LABORATOIRES BOIRON	herbal drops	200 ml
78		Teva Czech Industries	herbal drops	100 ml
79	PROPSAN	ENGELHARD ARZNEIMITTEL	herbal drops	100 ml
80	Original Schweden Bitter	RIVIERA HandelsGes m.b.H.	herbal drops	200 ml
81	Dr. Theiss Mucoplant drops with plantain and honey	Dr. Theiss Naturwaren GmbH	herbal drops	100 ml
82	Bronchipret ivy and thyme	BIONORICA SE	herbal drops	50 ml
83	Hedelix	Krewel Meuselbach GmbH	herbal drops	100 ml

**HERBAL TEAS
(23 samples)**

**LIQUID SAMPLES
(42 samples)**

**Oils (triacylglycerols,
fatty acids)**

Wide range of matrices

**CAPSULES
(19 samples)**

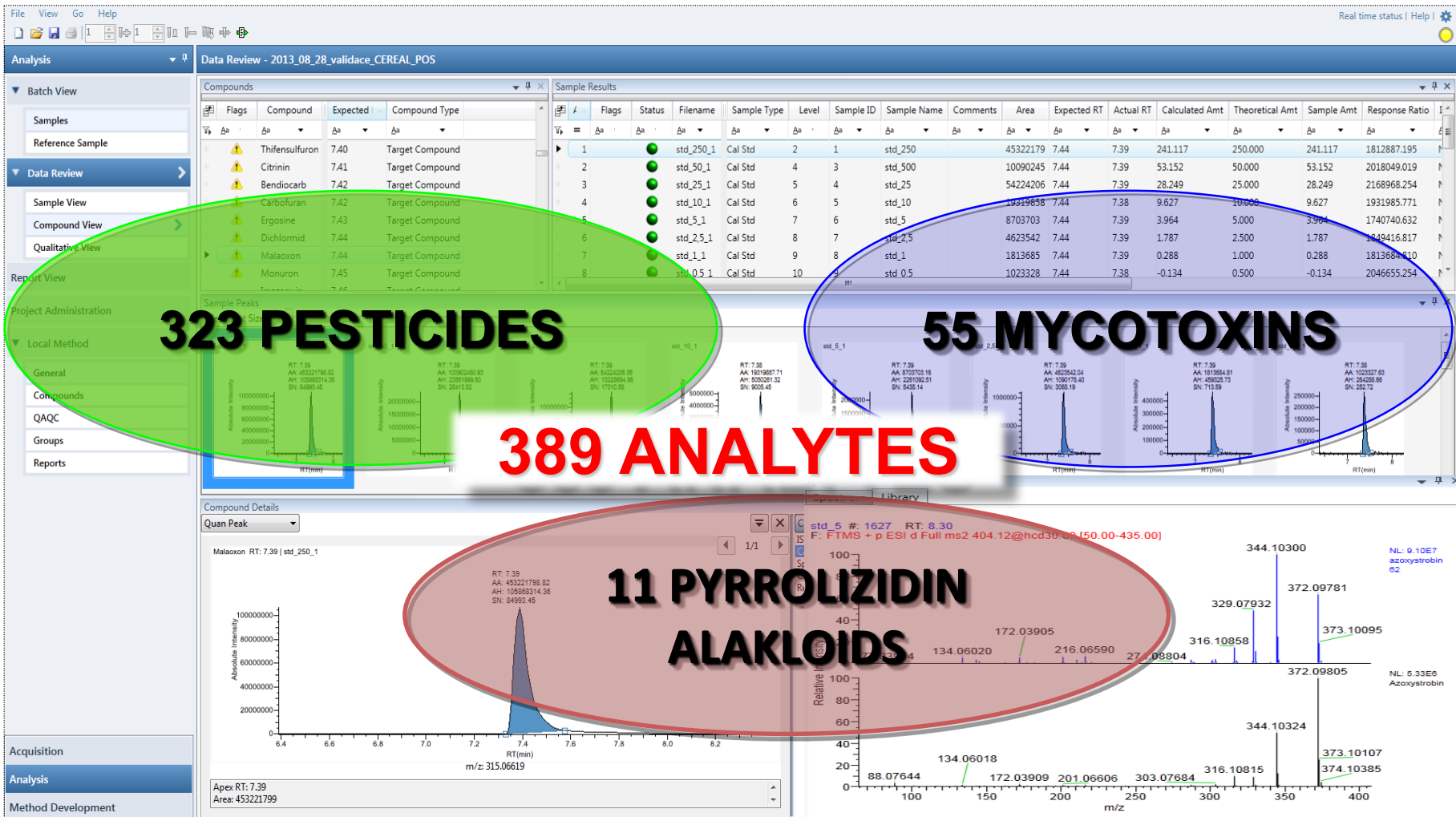
NO.	SAMPLE	PRODUCER	SAMPLE TYPE	PACKING
24	Apotheke Bio	Apotheke	capsules	150 g
25	HERBALMED Dr.Weiss		capsules	150 g
26	Milk thistle mixture		capsules	150 g
27	Revital ginkgo b		capsules	150 g
28	Redukta Gen		capsules	150 g
29	Liver detoxif		capsules	150 g
30	Livnorm		capsules	150 g
31	Silymar Plus		capsules	150 g
32	YUCCA SCHIDIGERA		capsules	150 g
33	YUCCA SCHIDIGERA 98,5%	HEW	capsules	150 g
34	Farmax milk thistle	SVUS Pharma	capsules	30 g
35	HERBALMED capsules	Simply You Pharmaceuticals, SWISS	pills	27,7 g
36	Milk thistle capsules	Róbert Hrabčák - ZEUS	capsules	60 g
37	GS Echinacea forte 600	Green-Swan Pharmaceuticals CR, a.s	pills	30 g
38	ROWATINEX	ROWA Pharmaceuticals Limited	capsules	10 ml
39	ROWACHOL	ROWA Pharmaceuticals Limited	capsules	10 ml
40	MENOFEM	BIONORICA SE	pills	60 tablet
41	Colpermin	Alliance Healthcare	capsules	20 tobolek
42	Broncipret thyme and primerose	BIONORICA SE	pills	20 tablet

**Phytoestrogens,
silymarin, antioxidants,
...**



Analytes on the list, HR MS/MS database established

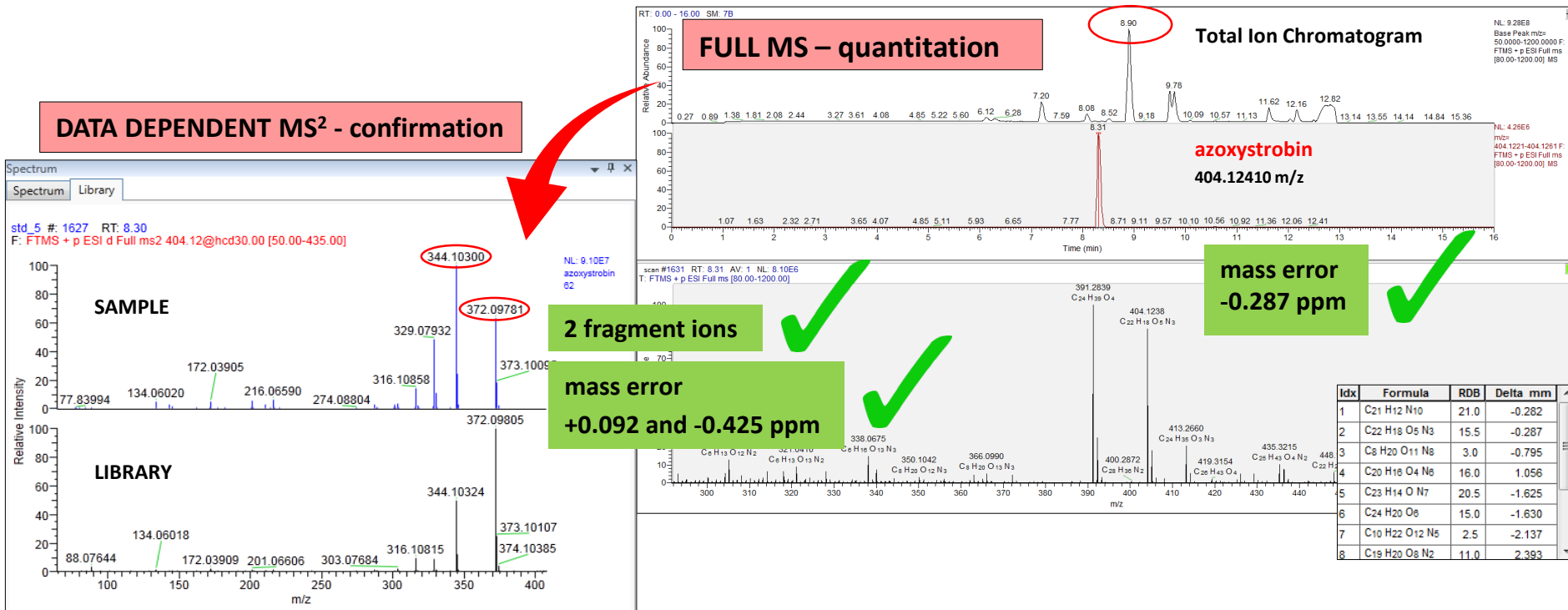
Thermo Trace Finder™ software



METHOD VALIDATION

- Two conceivable fragment ions were identified for 80.5% of target analytes
- All remaining analytes were confirmed by detection of at least 1 highly accurate (< 5 ppm) fragment ion

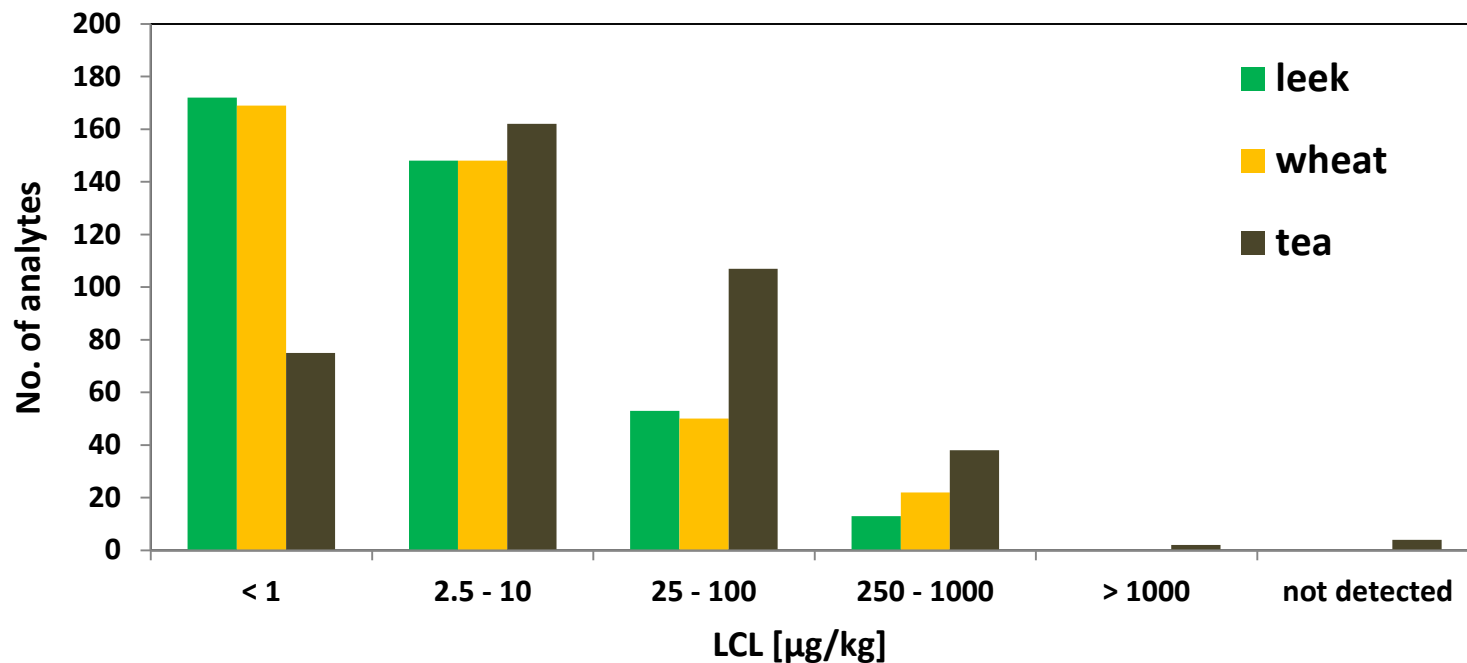
Example of chromatogram of **azoxystrobin** in contaminated sample



PERFORMANCE CHARACTERISTICS

■ Limits of quantification:

Method was validated for **herbal supplement** and also for matrices **wheat and leek**.

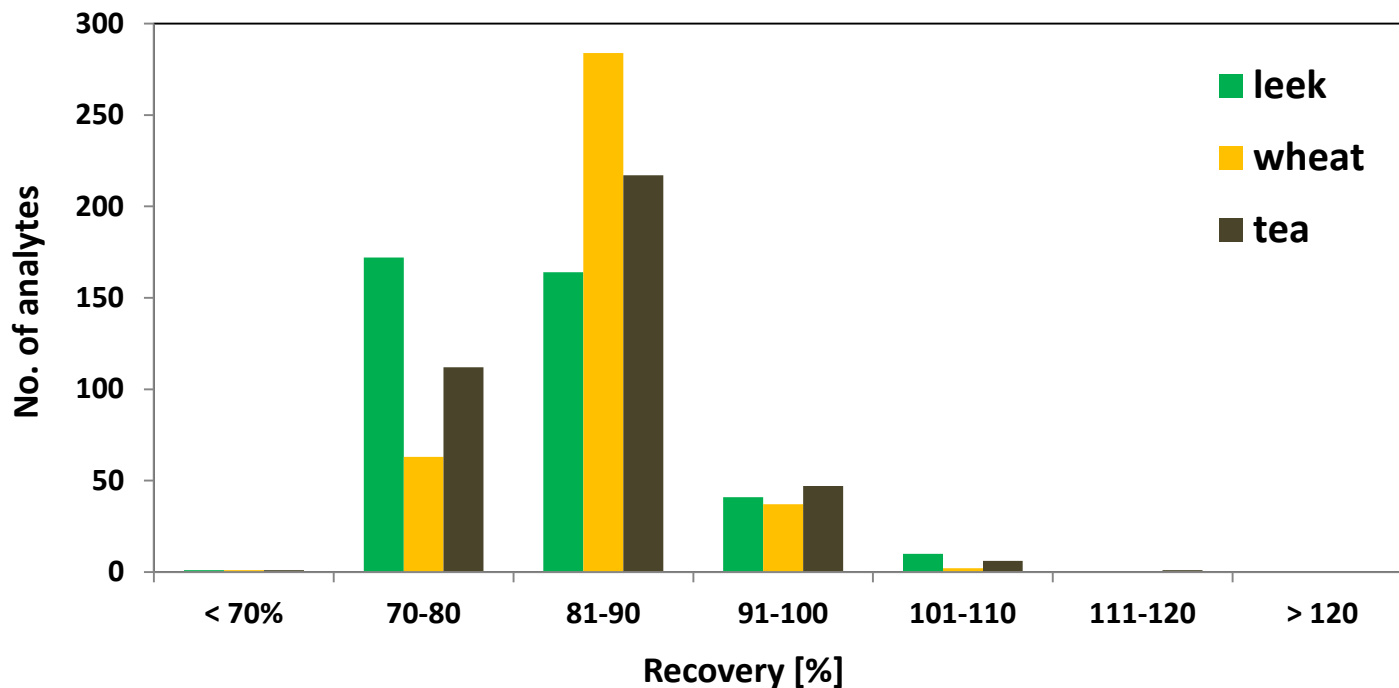


PERFORMANCE CHARACTERISTICS

■ Recovery:

Recoveries were in the range **70 – 120** for **99% of analytes**.

The only exception – polar deoxynivalenol-3-glucoside (around 40%).



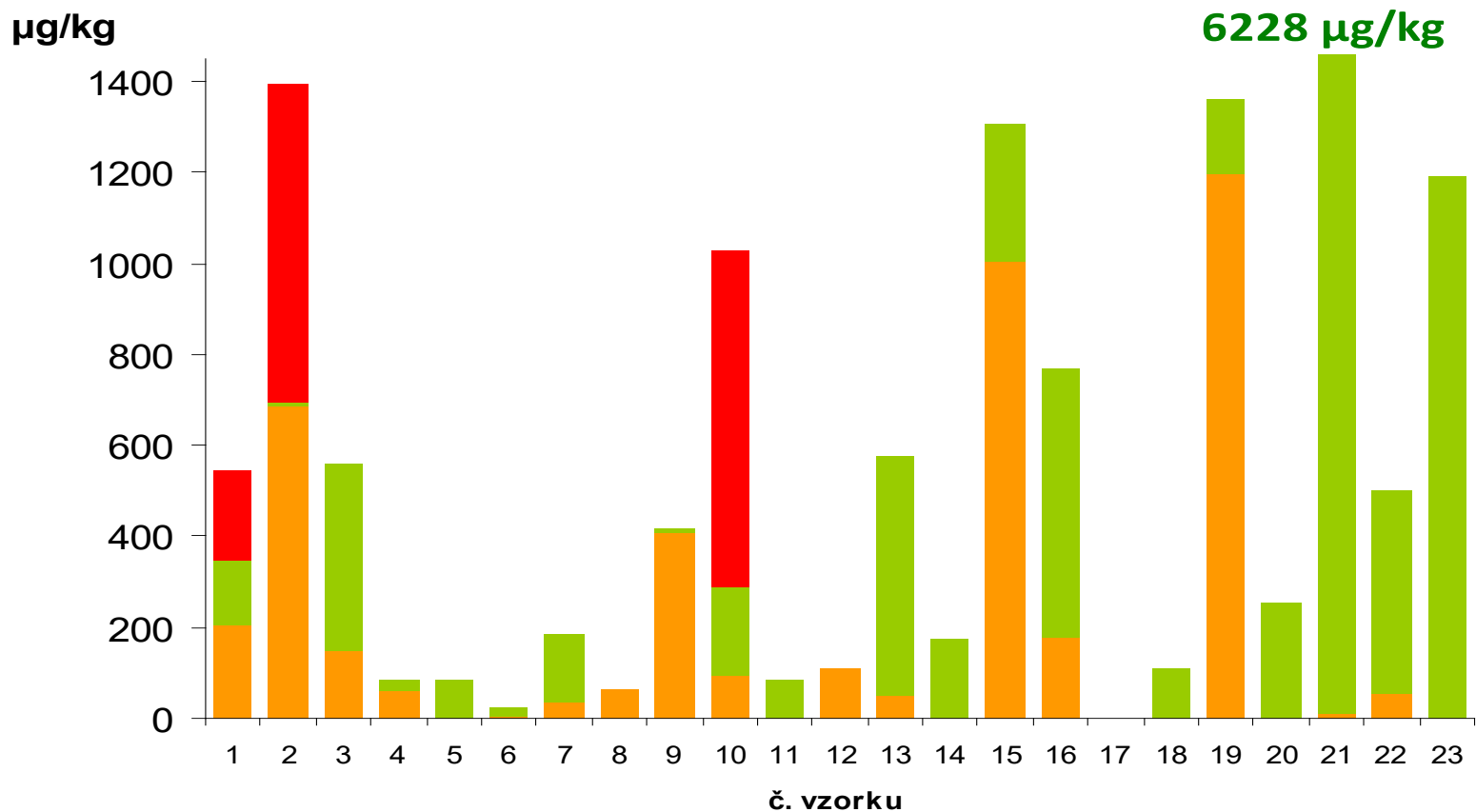
PERFORMANCE CHARACTERISTICS

■ Linearity, repeatability:

- 0.1 – 500 ng/mL 89% analytes
- 0.1 – 250 ng/mL 96% analytes
- 0.1 – 100 ng/mL 98% analytes

Matrix	R ²	RSD at LOQ [%]
tea	0.9880-0.9998	2.28-14.19
leek	0.9910-0.9999	0.56-13.73
wheat	0.9879-0.9999	0.25-9.38

Contamination of herbal tea samples



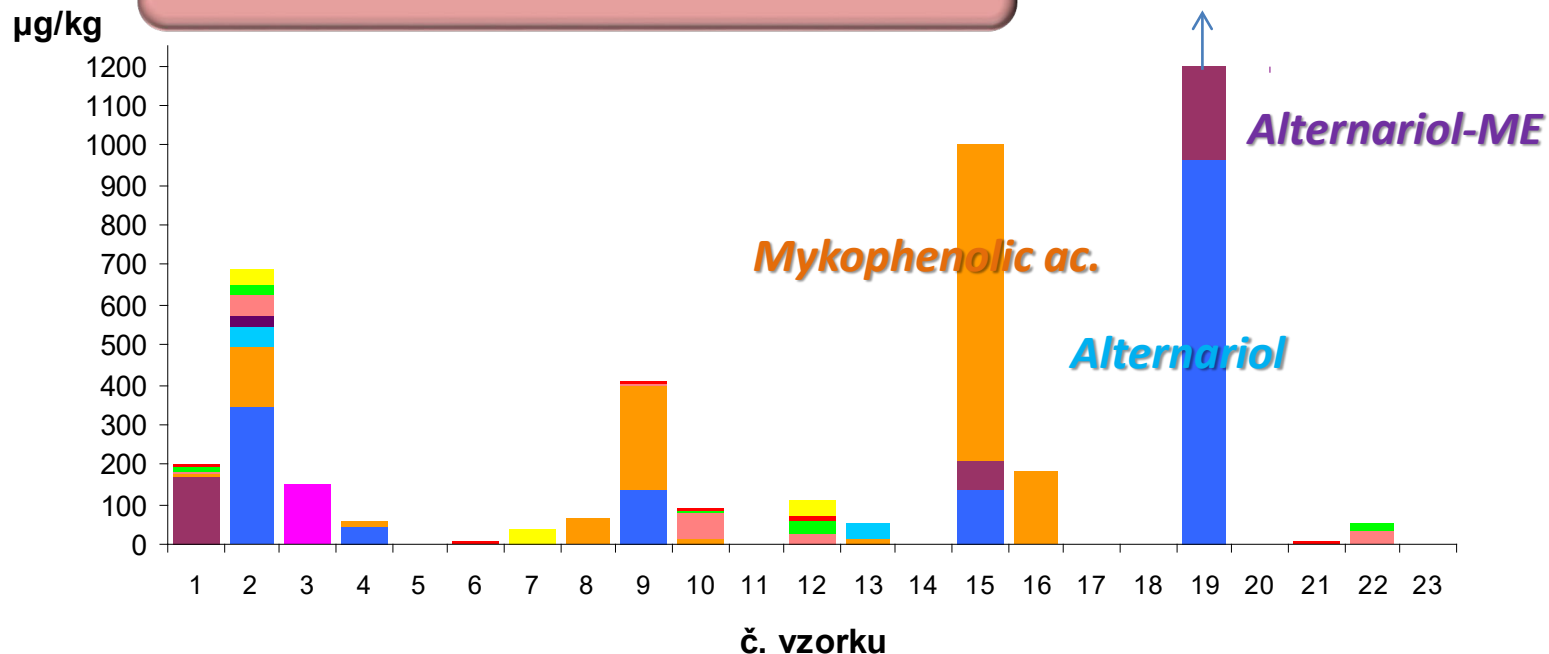
 Mycotoxins

 Pesticides

 Pyrrolizidine alkaloids

Mycotoxins

11 detected mycotoxins



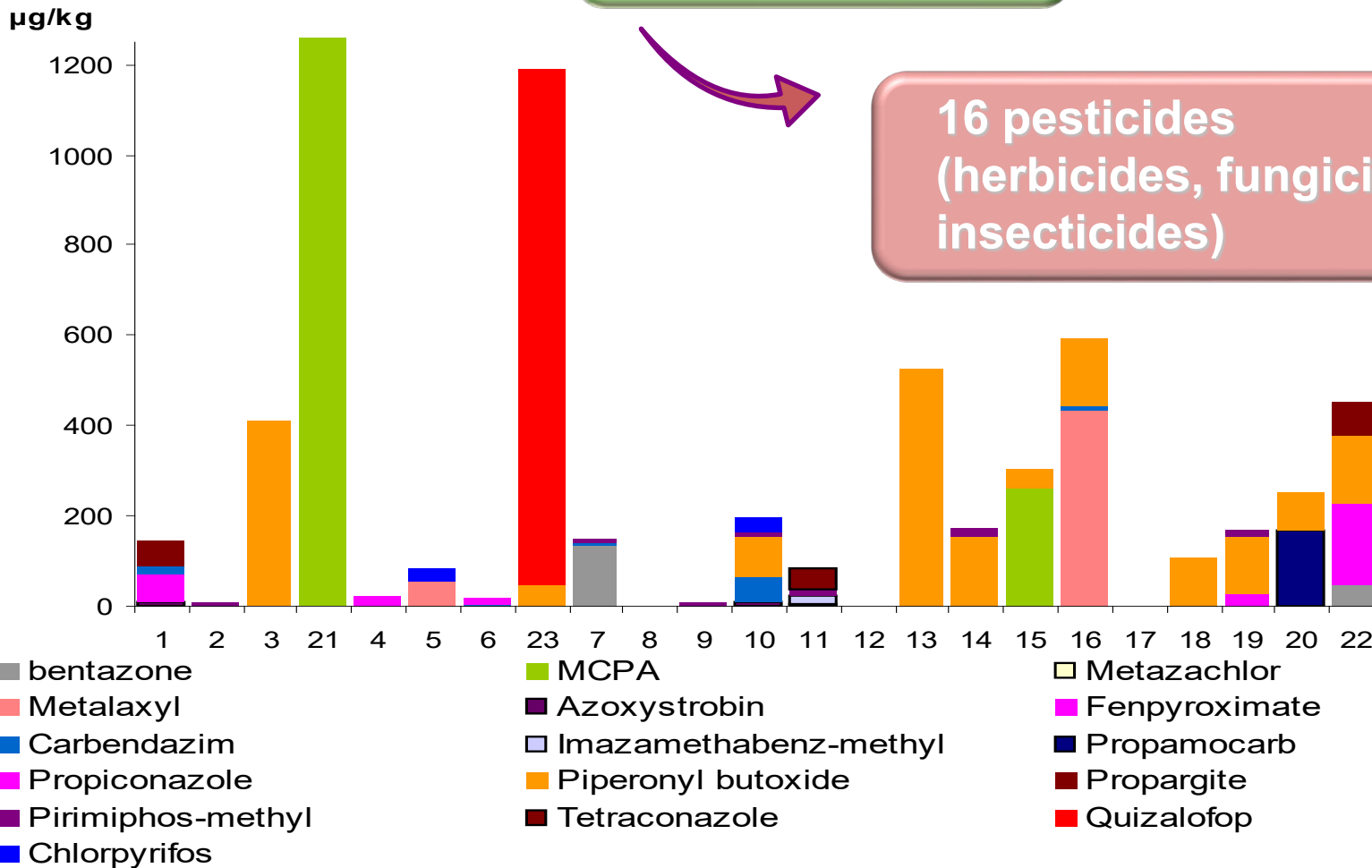
- alternariol
- alternariol-methylether
- Mycophenolic acid
- Diacetoxyscirpenol
- Ergotamine
- Enniatin B
- Enniatin B1
- Enniatin A1
- Enniatin A
- T-2 Toxin
- Beauvericin

Pesticides

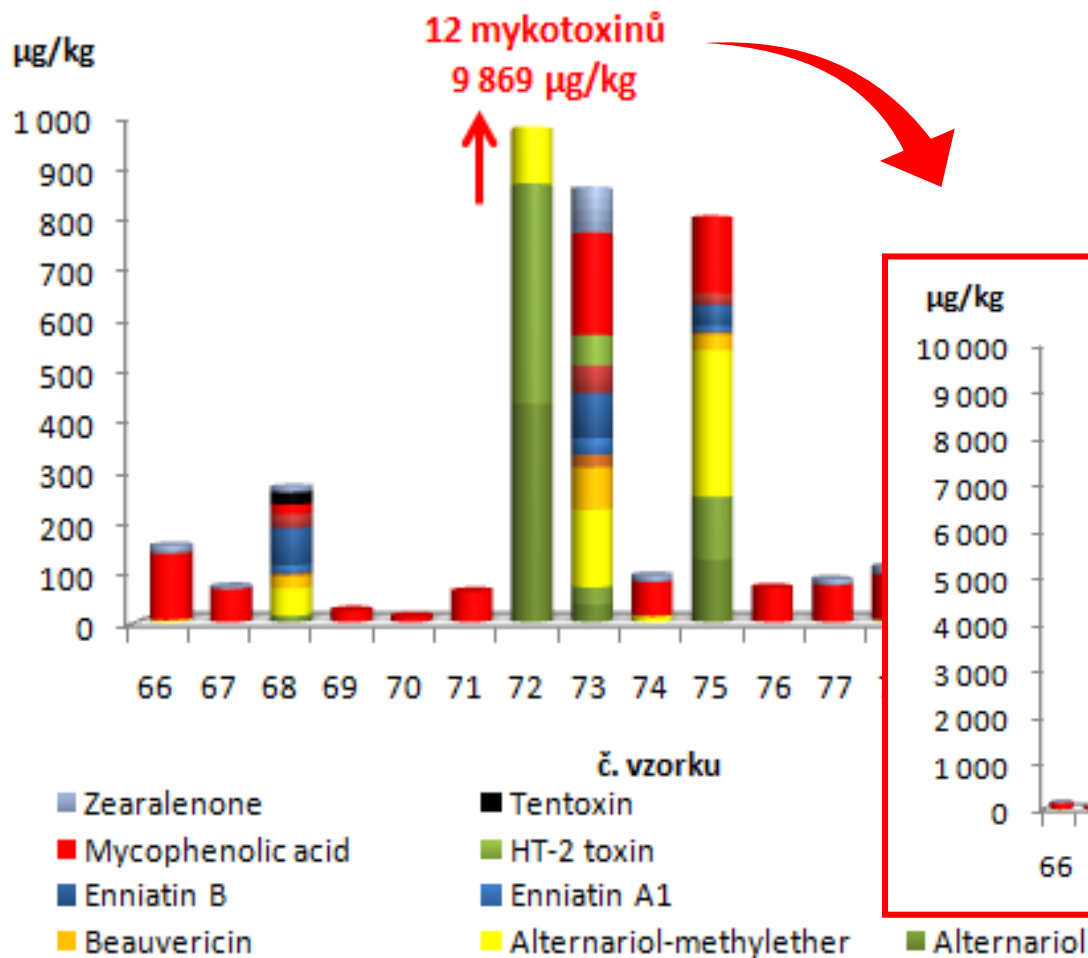
MCPA 5800 µg/kg,
carbendazim 4 µg/kg,
piperonyl butoxid 416 µg/kg

323 pesticides

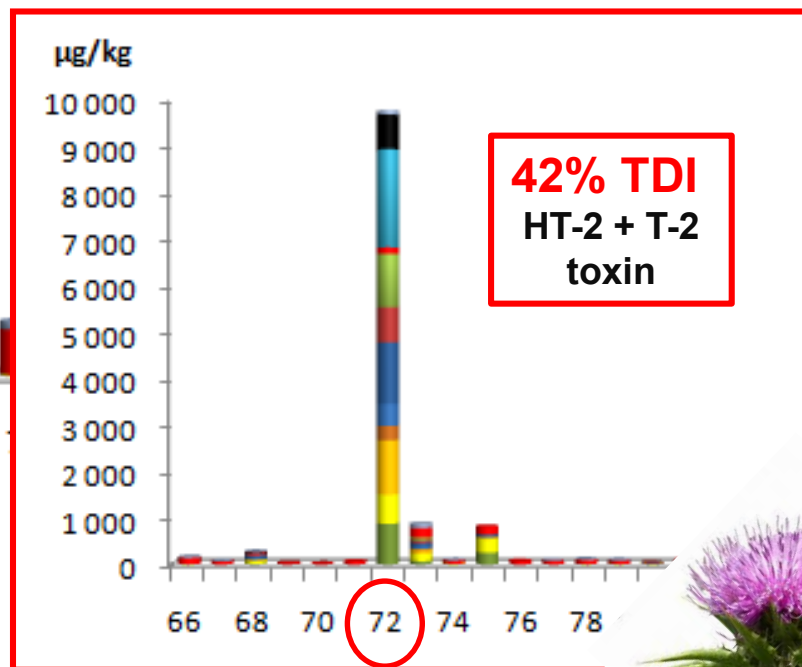
16 pesticides
(herbicides, fungicides,
insecticides)



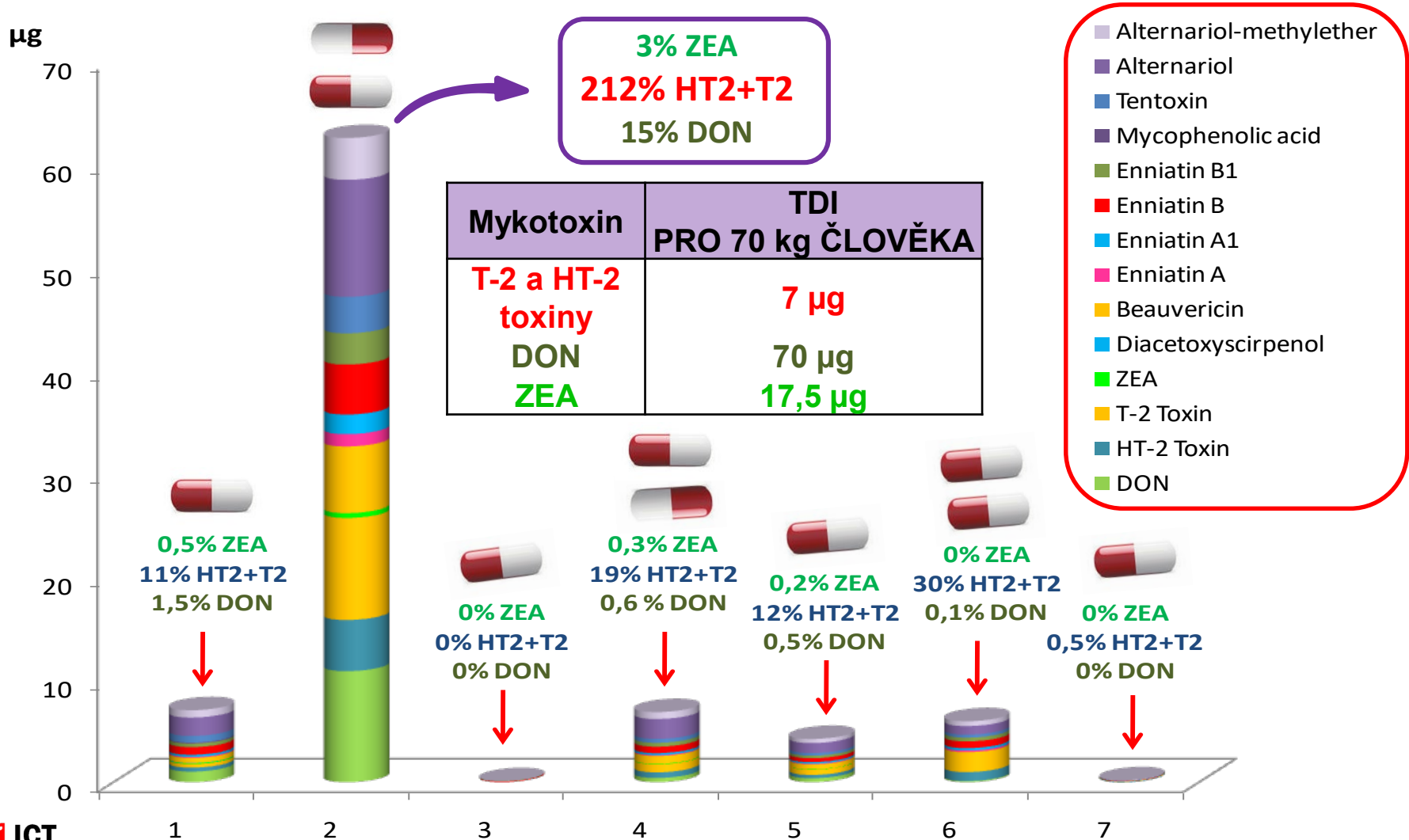
Mycotoxins in milk thistle-based dietary supplements



Mykotoxin	TDI 70 kg
T-2 a HT-2	7 µg
ZEA	17,5 µg



Mycotoxins in milk thistle-based dietary supplements fulfilling TDI (%)



Case study : **TARGET ANALYSIS**

**of mycotoxins and pesticide residue
in tea and spices collected at the Asian market**



Survey set-up

- Altogether, **420 samples** of different kinds of **tea (n = 120)** and **spices (n = 300)** collected at Asian market

No	Matrix	n	Matrix type
1	Green tea	40	tea
2	Oolong tea	40	
3	Black tea	40	
4	Black pepper	30	spices
5	White pepper	30	
6	Cardamom	30	
7	Coriander seed	30	
8	Cinnamon	30	
9	Cumin	30	
10	Chilli powder	30	
11	Nutmeg	30	
12	Star anise	30	
13	Turmeric powder	30	

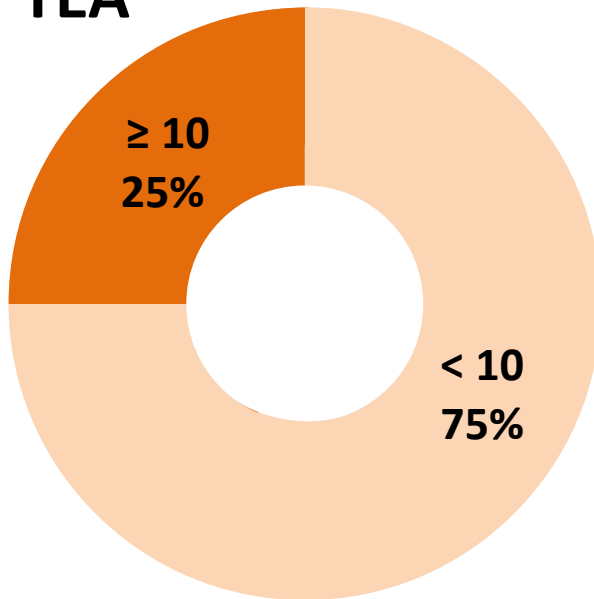
425
contaminants
analyzed

- 357 pesticides**
- 57 mycotoxins**
- 11 pyrrolizidine alkaloids**

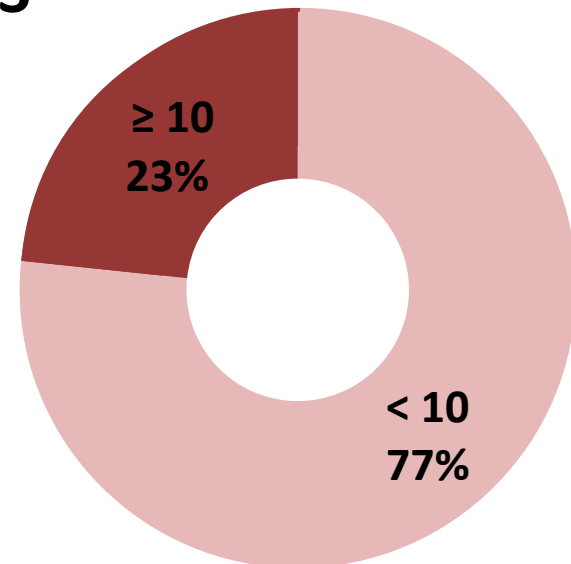
Co-occurrence of pesticide residues and mycotoxins in analyzed samples

- Only 6 % of samples showed no contamination (mainly turmeric)
- Frequent occurrence of multiple contaminants in the majority of samples

TEA

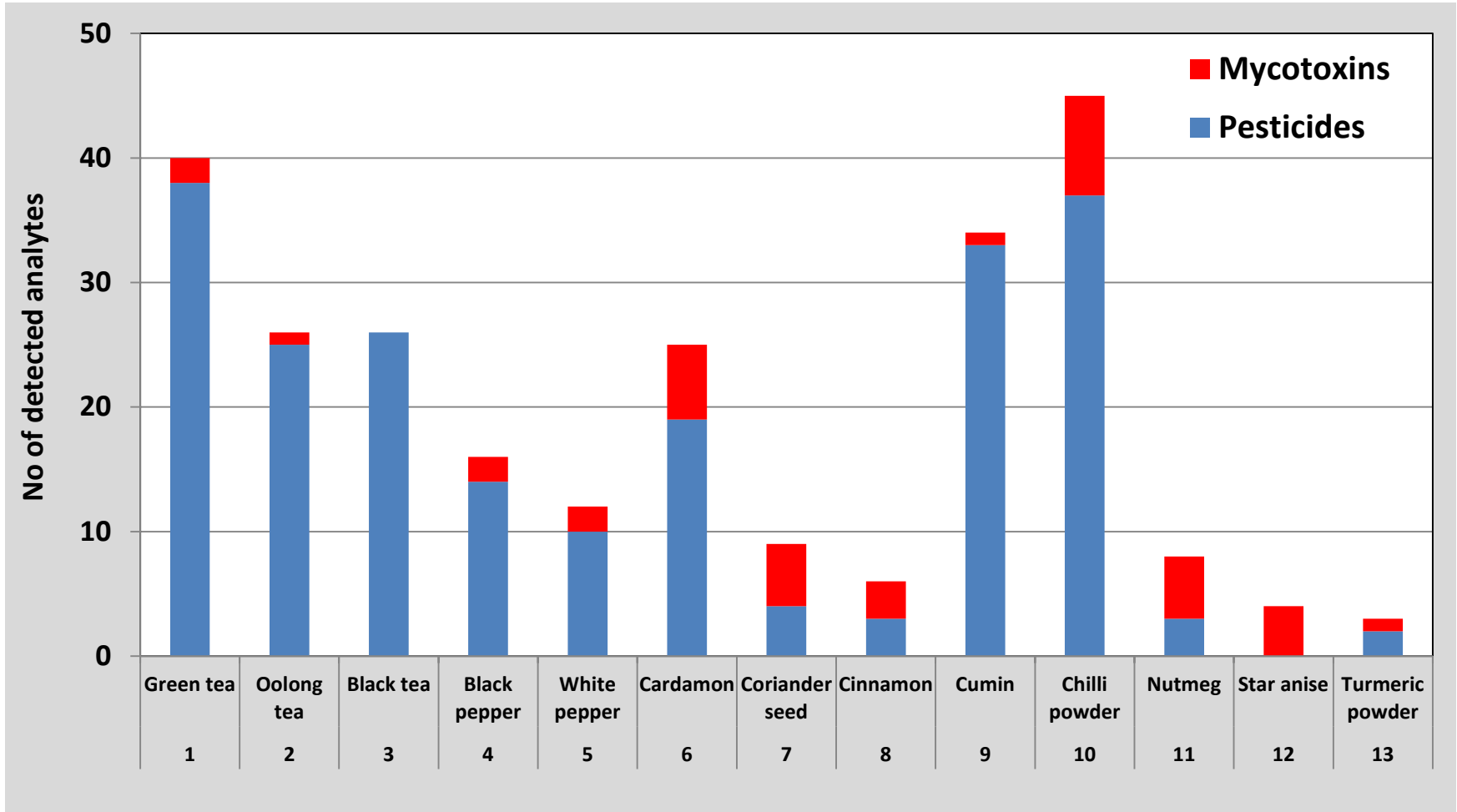


SPICES



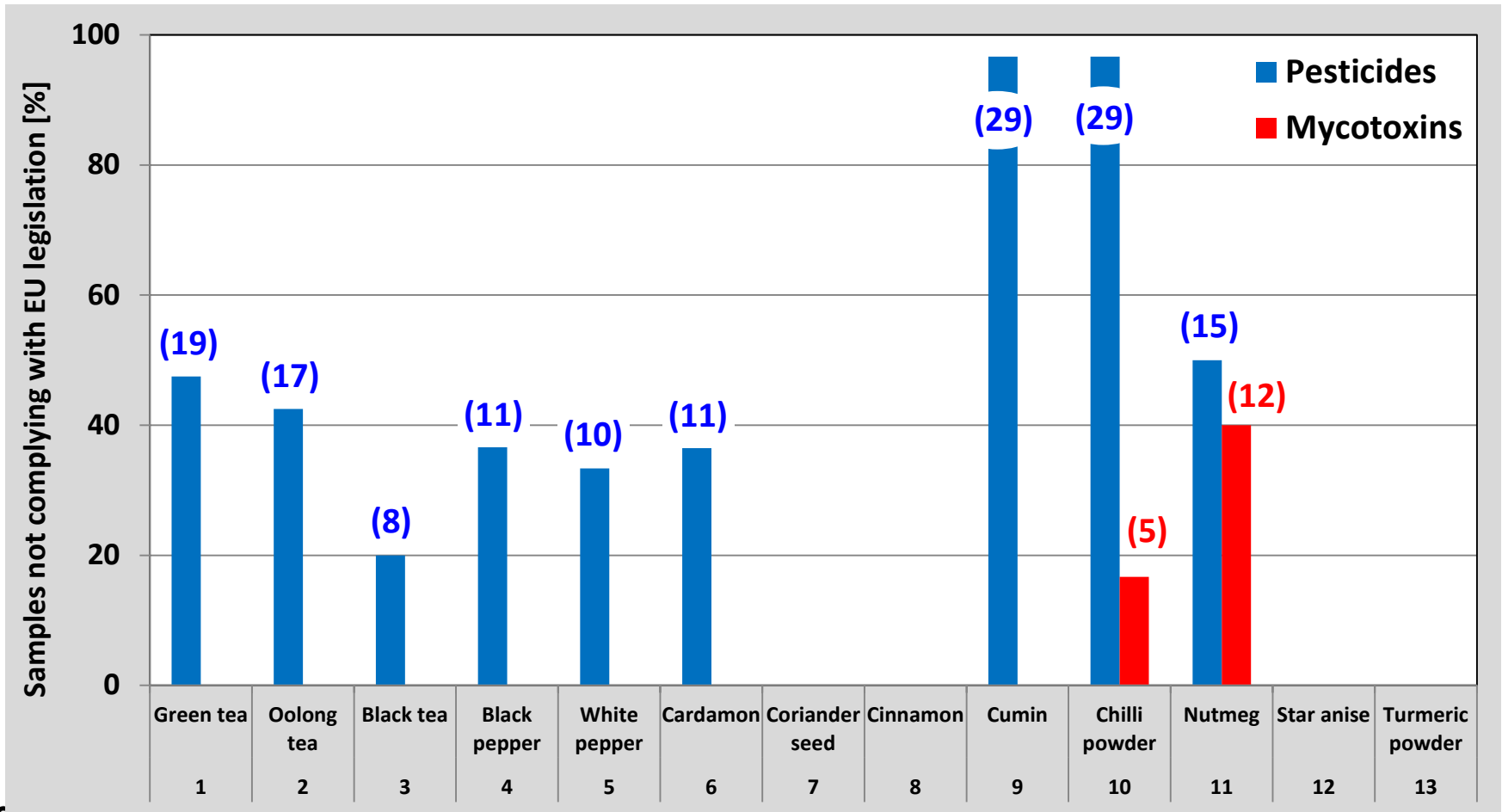
Results – summary (1)

- Number of detected analytes



Results – summary (2)

- Percentage of samples **not complying with EU legislation**
(*number of samples in



Green tea (n = 40)



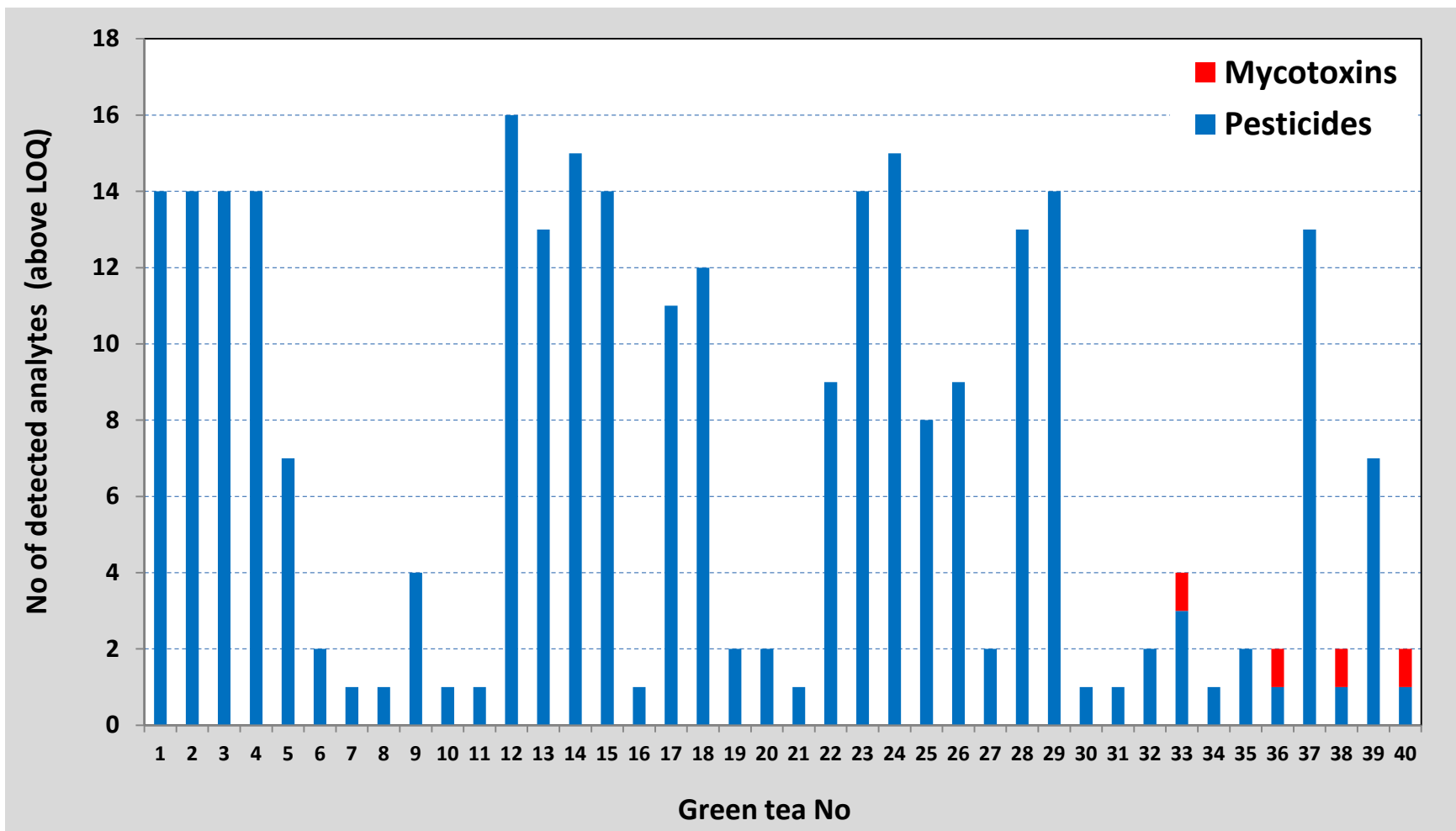
- In summary, **HIGH CONTAMINATION WITH PESTICIDE RESIDUES** was observed. High incidence of multi-contaminated samples.
- Detected analytes:
 - Pesticide residues 38
 - Mycotoxins 2
- All samples (n = 40) were positive at least for 1 analyte.
- 19 samples (48 %) would not comply with EU legislation:
 - Tebuconazole (14 samples), flubendiamid (11x), lufenuron (11 x), chlorantraniprole (9x), flonicamid (7x), acetamiprid (7x)...



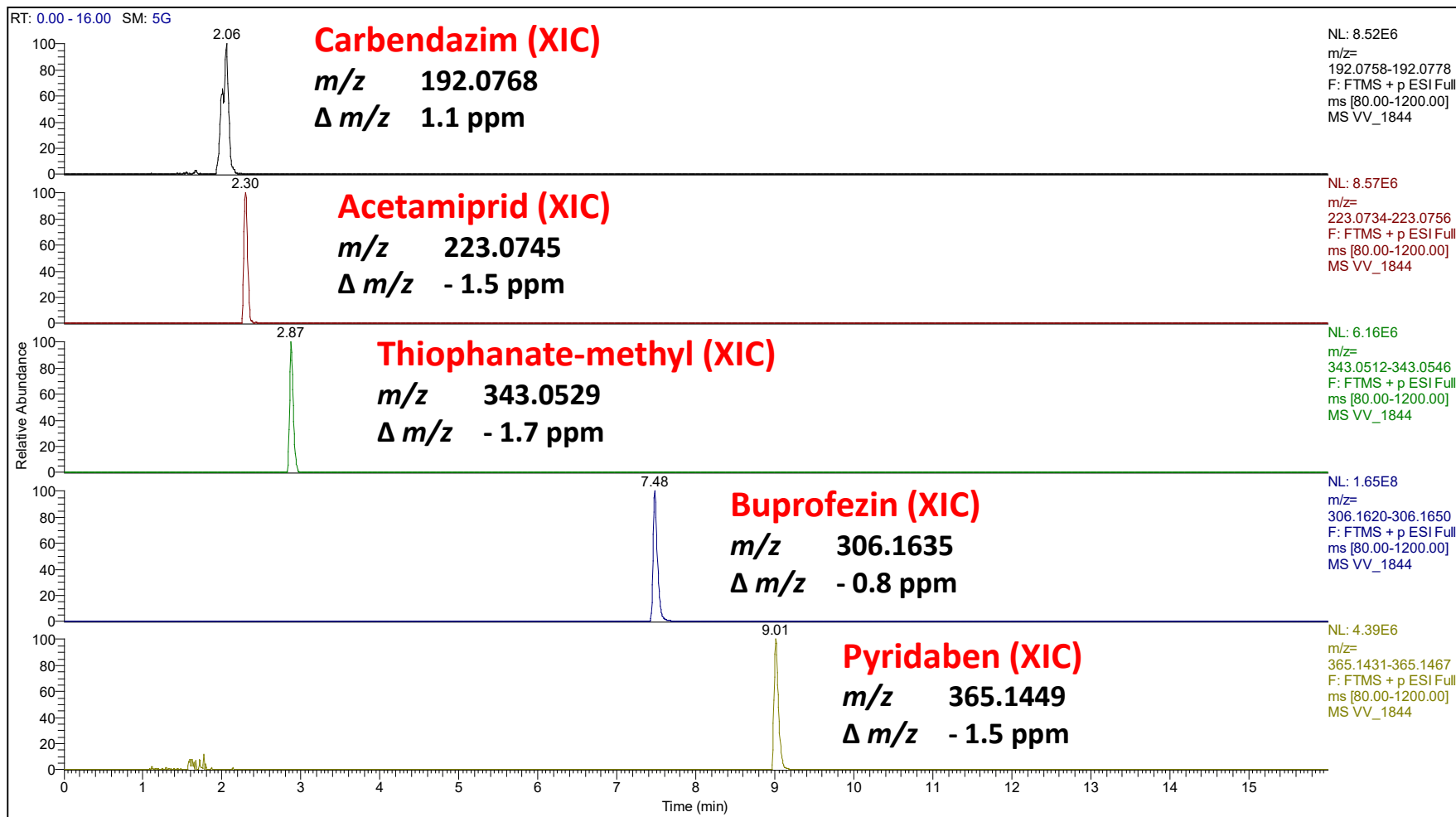
Green tea (n = 40)



Number of detected analytes



Green tea – the example of LC-HRMS analysis of highly contaminated sample



Black tea (n = 40)

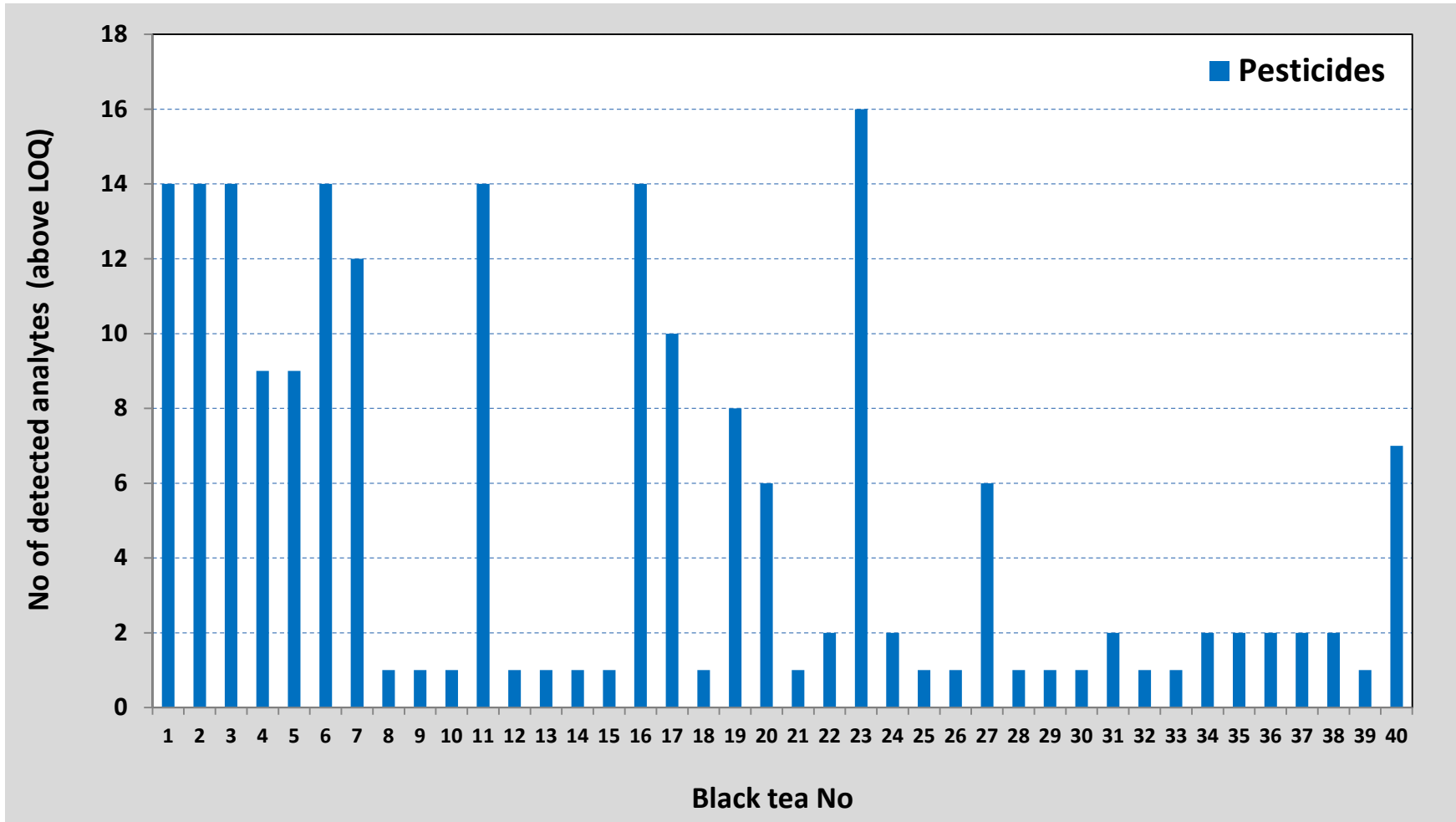


- **MODERATE/HIGH CONTAMINATION WITH PESTICIDE RESIDUES** was observed. High incidence of multi-contaminated samples.
- Detected analytes:
 - Pesticide residues 26
- All samples (n = 40) were positive at least for 1 analyte.
- 8 samples (20 %) would not comply with EU legislation:
 - Acetamiprid (7 samples), carbendazim (1x).

Black tea (n = 40)



- Number of detected analytes



Black pepper (n = 30)

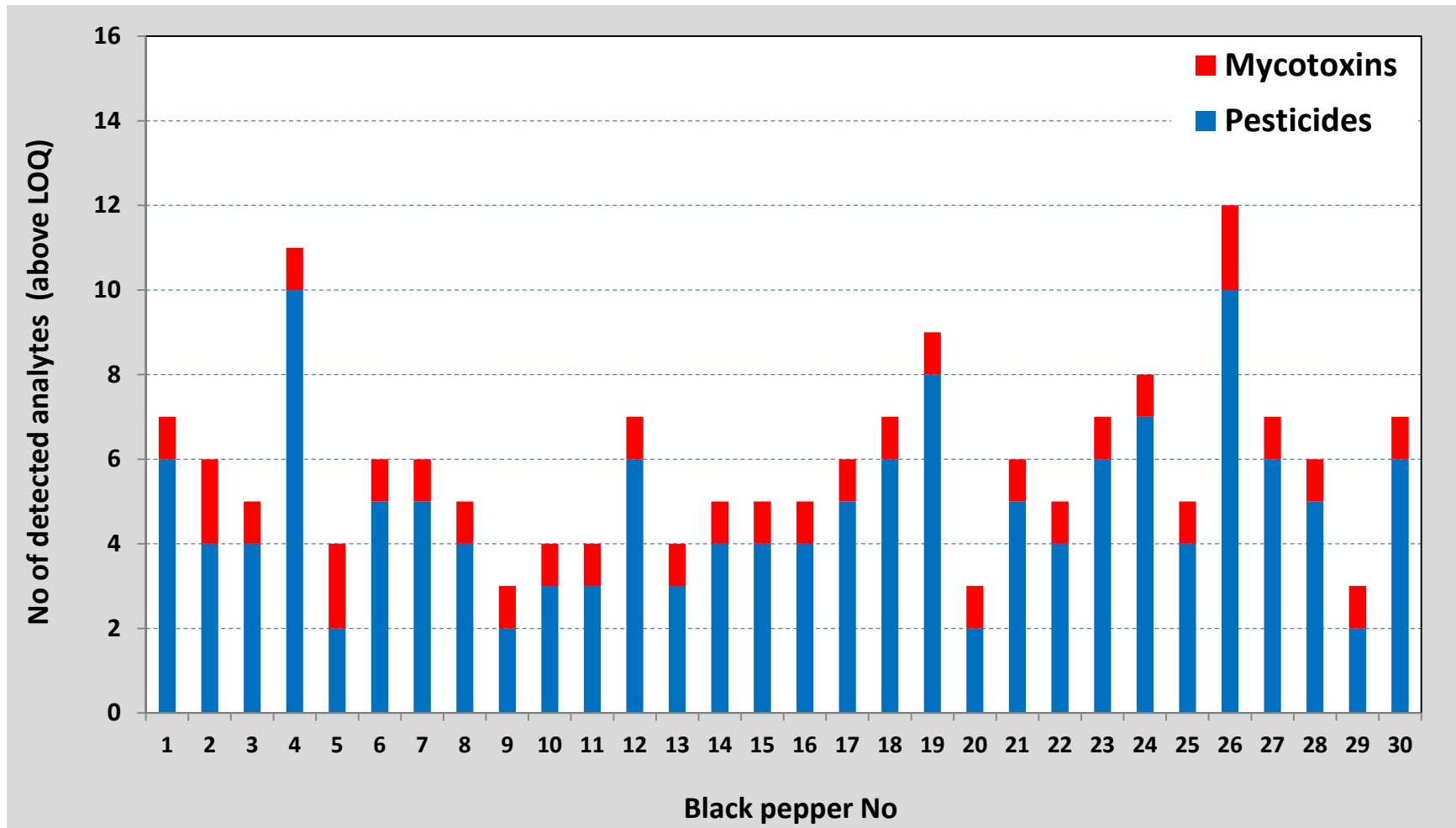


- **MODERATE/HIGHER CONTAMINATION WITH PESTICIDE RESIDUES** was observed. High incidence of multi-contaminated samples.
- Detected analytes:
 - Pesticide residues 14
 - Mycotoxins 2
- All samples (n = 30) were positive at least for 3 analytes.
- 11 samples (37 %) would not comply with EU legislation:
 - Imidacloprid (8 samples), carbendazim (4x), propamocarb (3x), metalaxyl (3x), cypermethrin (3x), fipronyl (3x)...

Black pepper (n = 30)



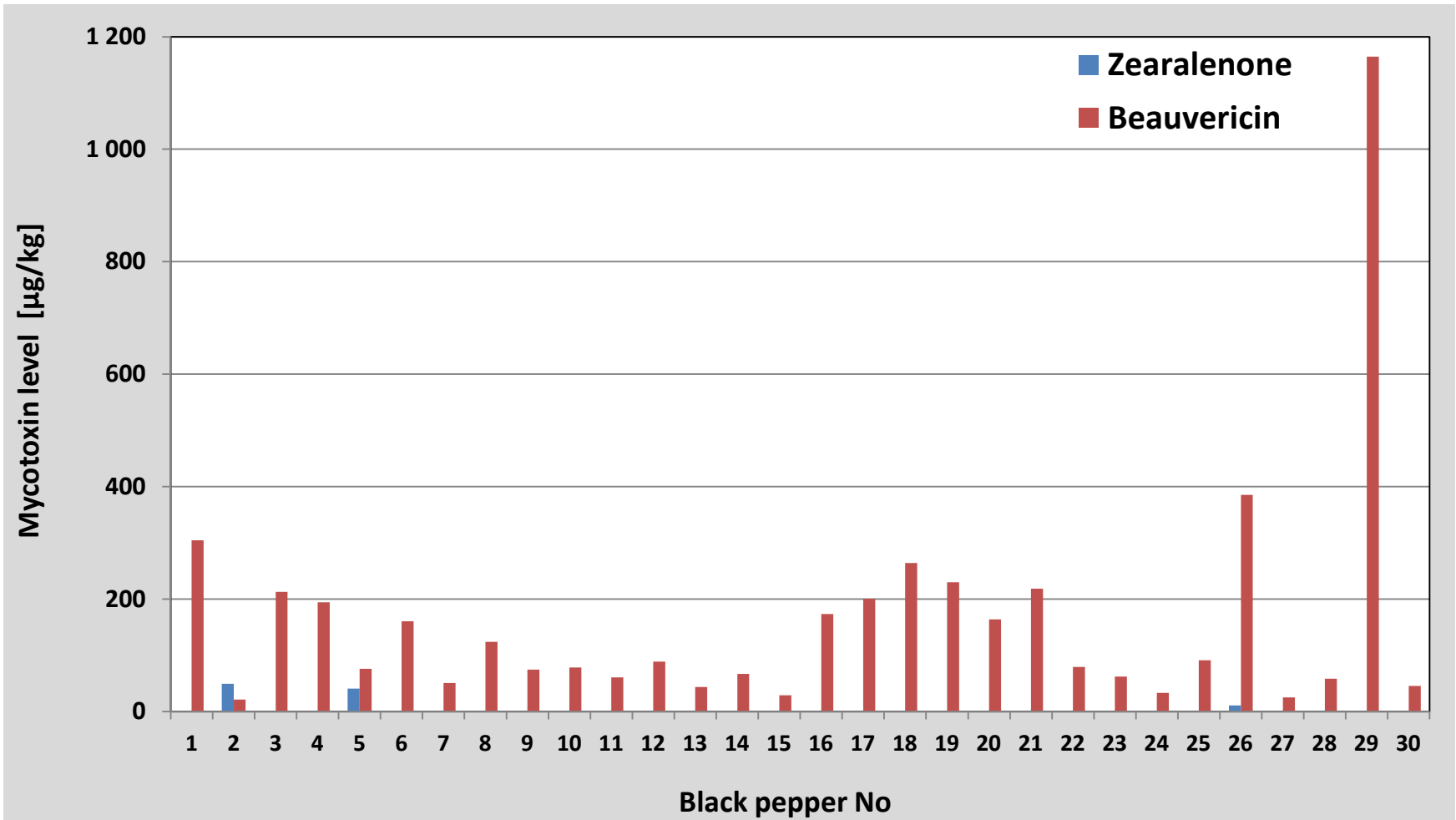
- Number of detected analytes



Black pepper (n = 30)



- Detected **mycotoxins** [$\mu\text{g}/\text{kg}$]



Chilli powder (n = 30)



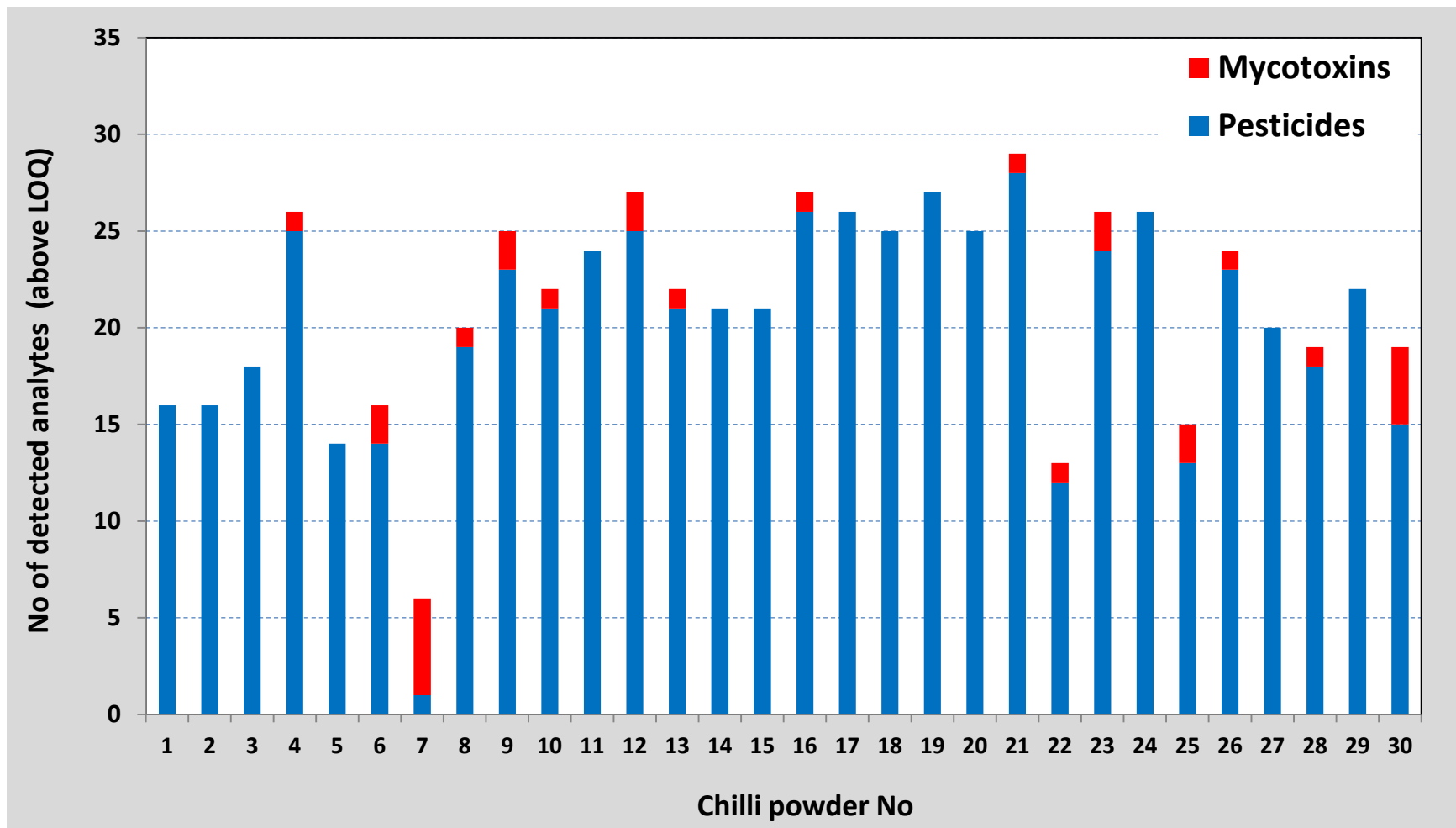
- **VERY HIGH CONTAMINATION WITH PESTICIDE RESIDUES** and also **MYCOTOXINS** was observed. High incidence of multi-contaminated samples.
- Detected analytes:
 - Pesticide residues 37
 - Mycotoxins 8
- All samples (n = 30) were positive at least for 6 analytes.
- 29 samples (97 %) would not comply with EU legislation:
 - Triazophos (25 samples), imidacloprid (22x), acetamiprid (19x), fipronyl (18x)...
 - Aflatoxin B1 (5 samples), ochratoxin A (1x)



Chilli powder (n = 30)



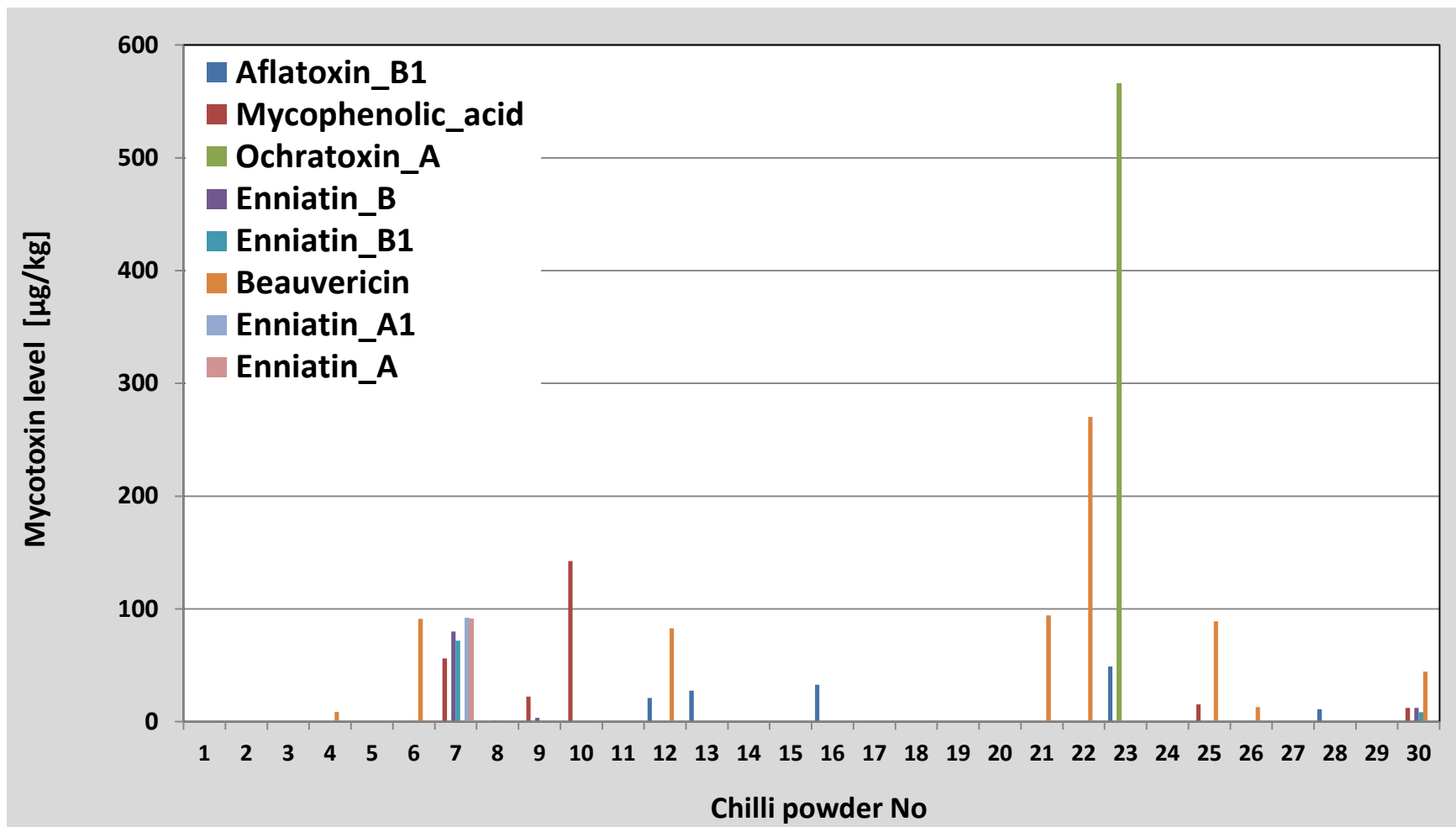
- Number of detected analytes



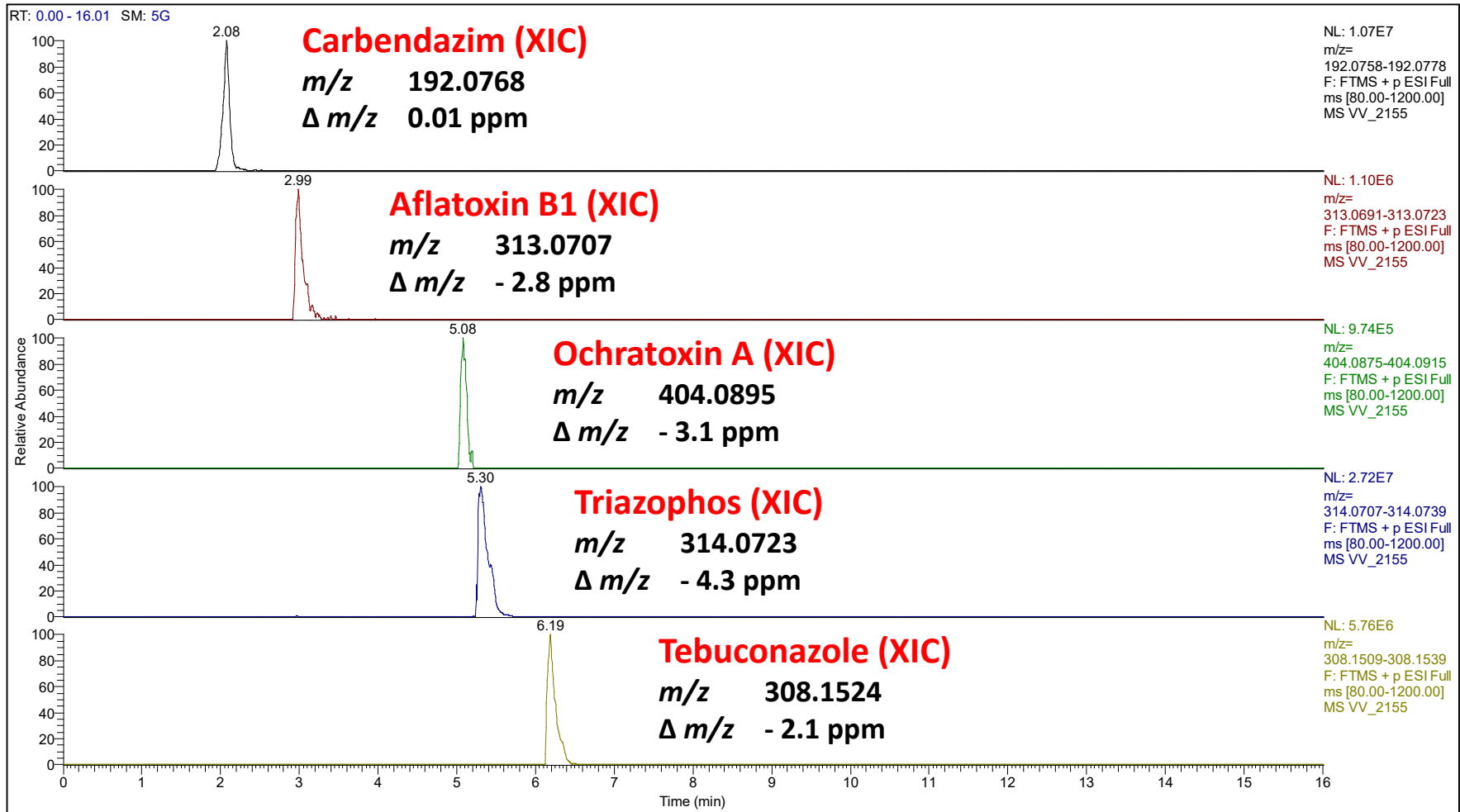
Chilli powder (n = 30)



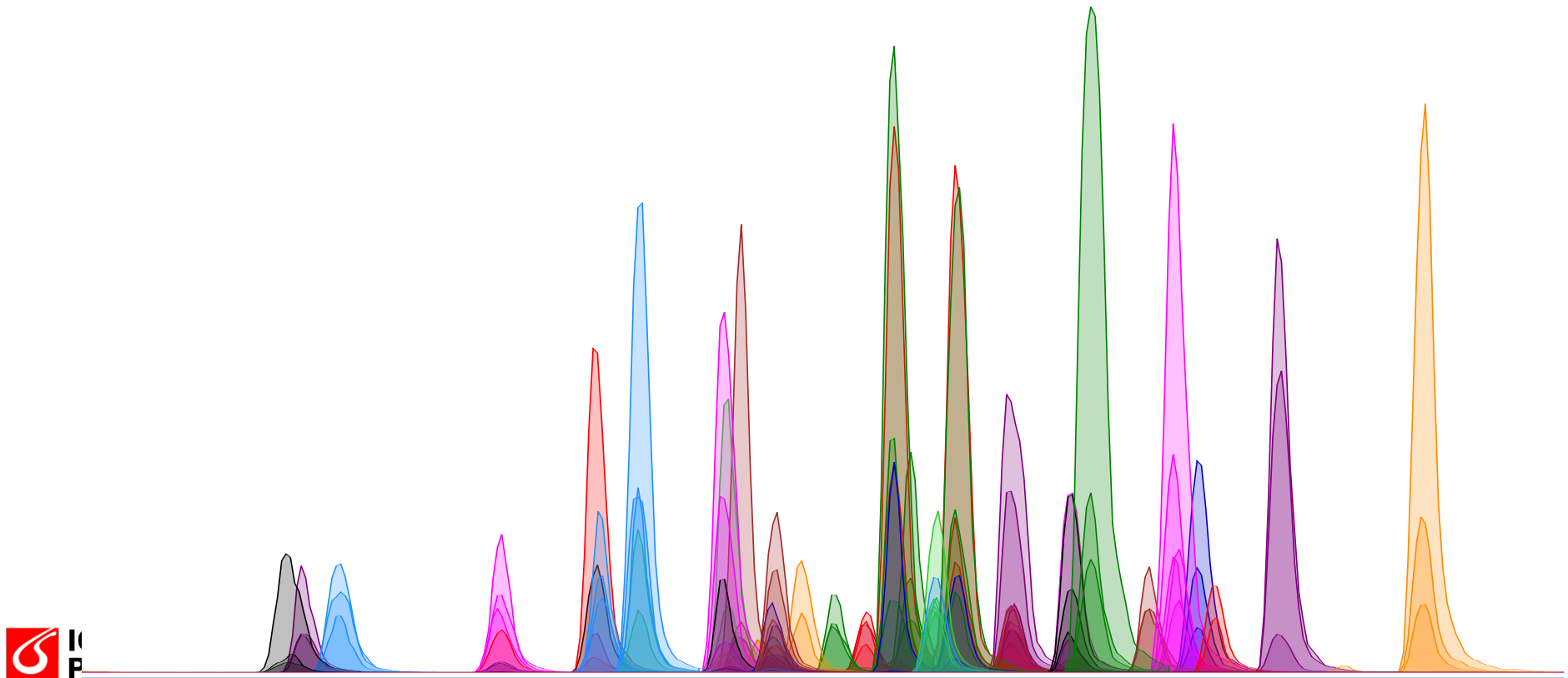
- Detected **mycotoxins** [$\mu\text{g}/\text{kg}$]



Chilli powder – the example of LC-MS/MS analysis of highly contaminated sample



Thank you very much for your kind attention



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