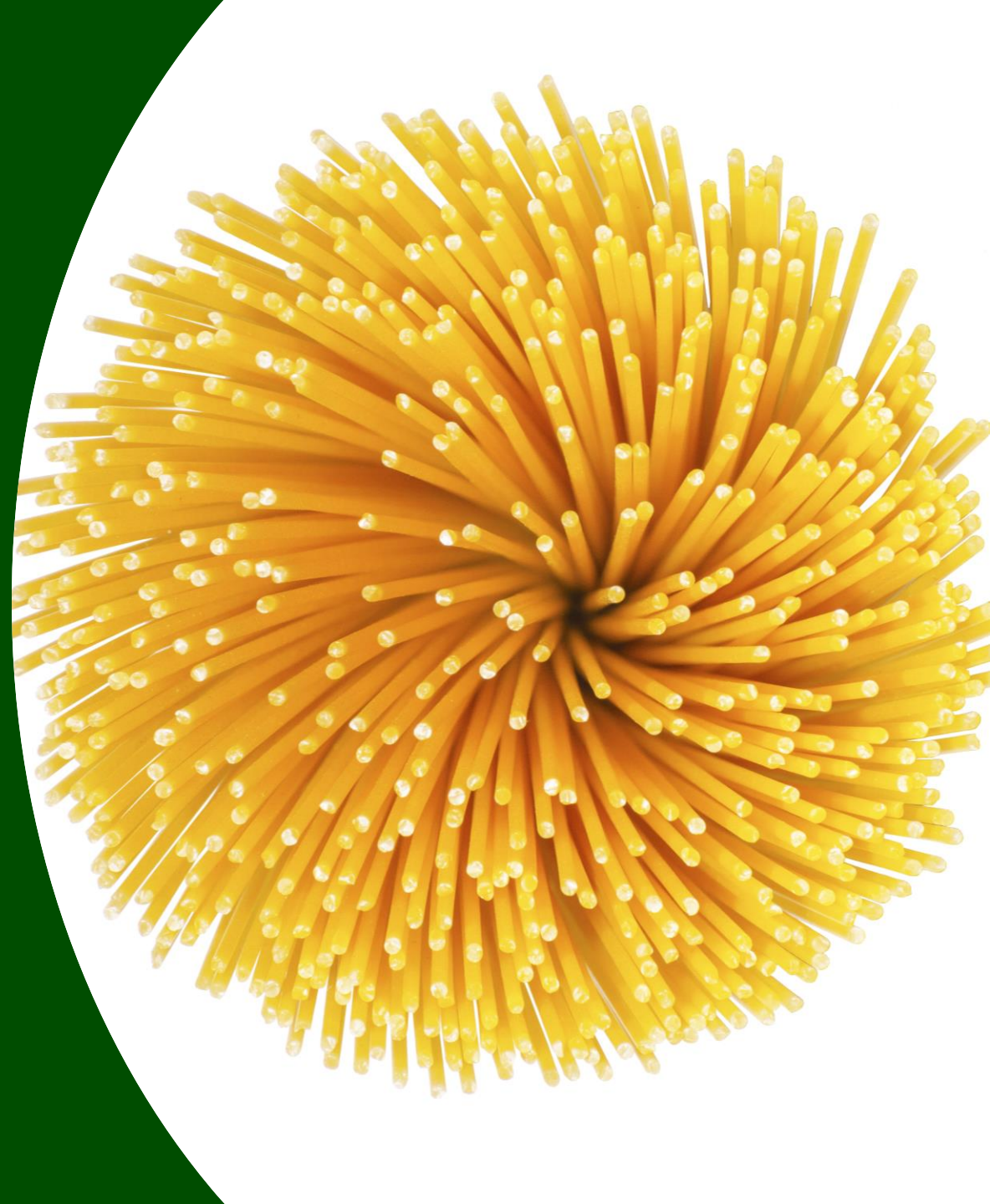


Comprehensive pesticide monitoring in food and feed:

Integrating targeted and untargeted analysis

Ivan Aloisi, Paul Zomer, Hans Mol
Wageningen Food Safety Research (WFSR)



WFSR in short



One of the 8 research institutes of WUR

Topic: food safety



Co-workers

> 400 including PhD students, interns and foreign guests



History

Over 130 years



80% for
**Ministry of Agriculture,
Fisheries, Food Security
and Nature and the NVWA**
(authority for food and
consumer products)



Located

on the Wageningen Campus



Our activities



Reference institute



Method development



Measuring and detecting substances



Safe food production



Effects of substances on humans and animals



Training and consultancy



Food fraud and composition



24/7

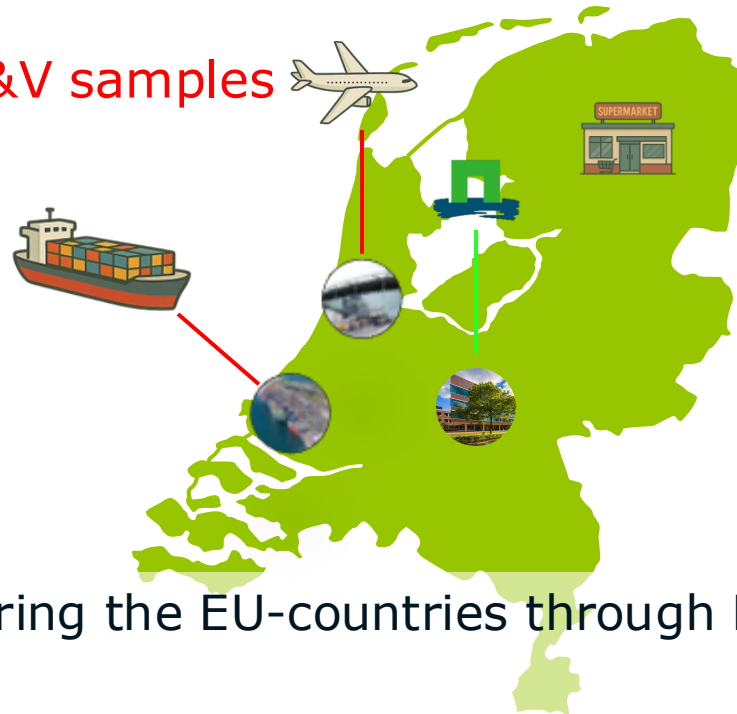
Pesticide team at WFSR

National Reference Laboratory (NRL) for pesticide residues in food and feed



Fresh F&V samples

Oranges and other products:
(peanuts, rice, oilseeds or
spices, etc.)



Import control: Samples entering the EU-countries through Rotterdam and Amsterdam

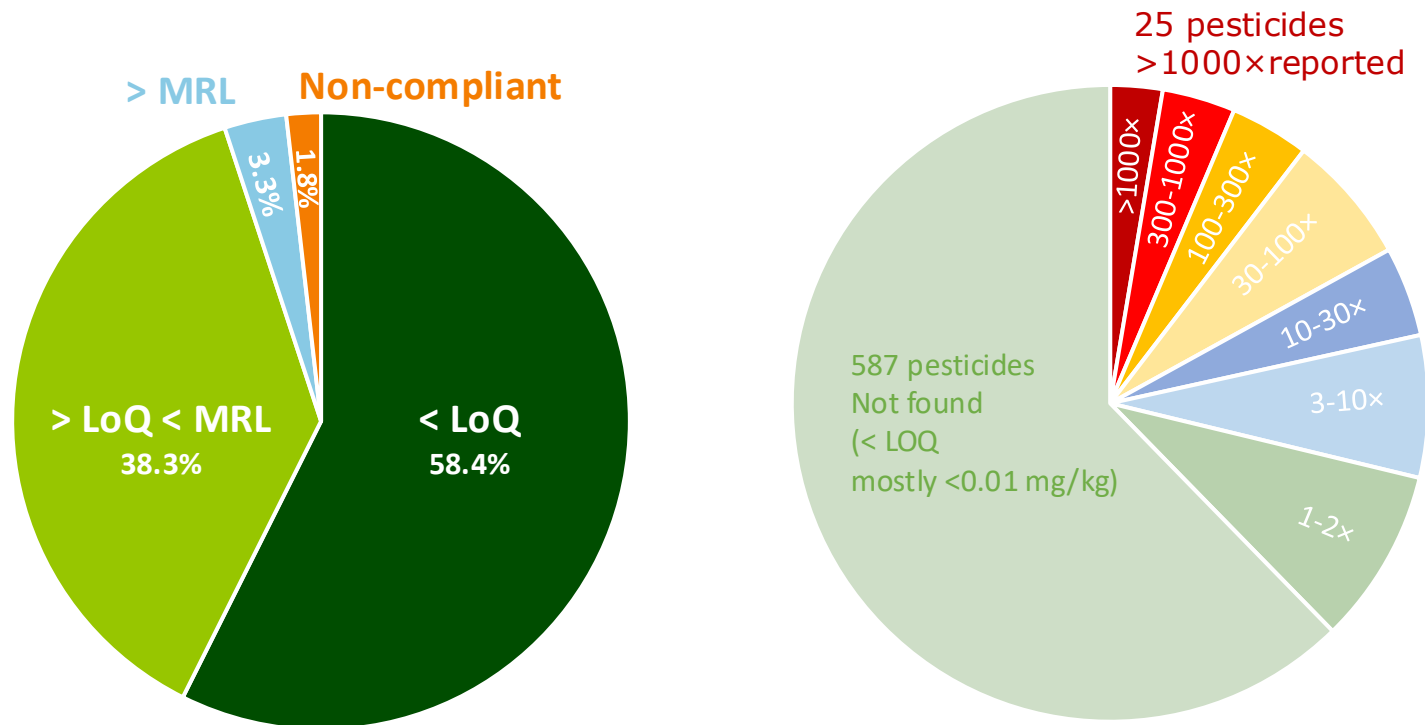
Routine monitoring: For the different stores (whole sales and retails) all over the Netherlands

Research and method development: To improve methods and increase the pesticides coverage

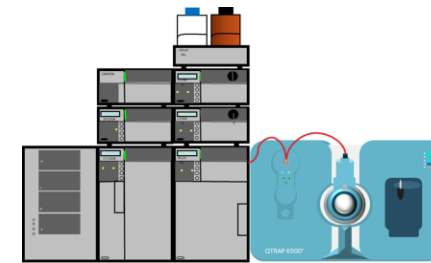
The challenge of comprehensive pesticide analysis

More than 2,000 pesticides (+ metabolites) with diverse physicochemical properties require broad-spectrum analytical methods

EFSA report*: 86,449 samples of food products and up to 942 different pesticides



Current approach at WFSR



LC-MS/MS
~ 300 pesticides



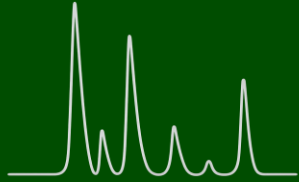
GC-MS/MS
~ 200 pesticides

Targeted measurement of 500 out of 2,000 pesticides = **Coverage Gaps!**

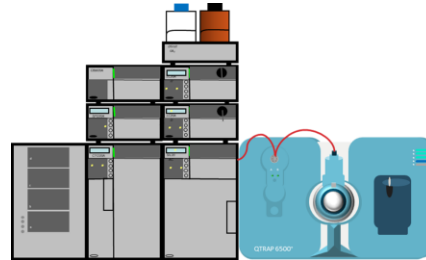
Instruments comparison

How to increase the coverage? From triple quadrupole to high-resolution mass spec

Chromatography
coupled to
Mass spectrometry



LC-MS/MS
GC-MS/MS



LC-HRMS
GC-HRMS



Sensitivity



+++

>

++

Selectivity



+++
(selective transitions)

≧

+++
(accurate masses)

Scope



Target quant of known
compounds
(~300 compounds)

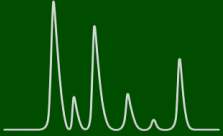
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Target quant
Suspect screening
Unknown screening



Strategies comparison

How to increase the coverage? Target, suspect, non-target screening

	Confirmatory/quant Target-screening	Suspect-screening	Non-target screening
Sample preparation & Data acquisition	Optimized Generic	Generic	Generic
Reference Standards injection	Every batch At least once for t_r	Not available	Not available
Data processing	Specific 2 ions and t_r	Database and mass spectral libraries	Mass spectral libraries and differential analysis
Detected pesticide	Quantitative Confirmatory analysis is needed	Confirmatory analysis is needed	N/A
Validation	Full validation: LOQ Full Validation: SDL (SANTE/11312_2021-V2026)	N/A	N/A

MS/MS

HRMS



The power of HRMS

Extracted ions chromatograms (EIC): target and suspect screening

Target/suspect screening

Compound => formula => exact mass (Da)

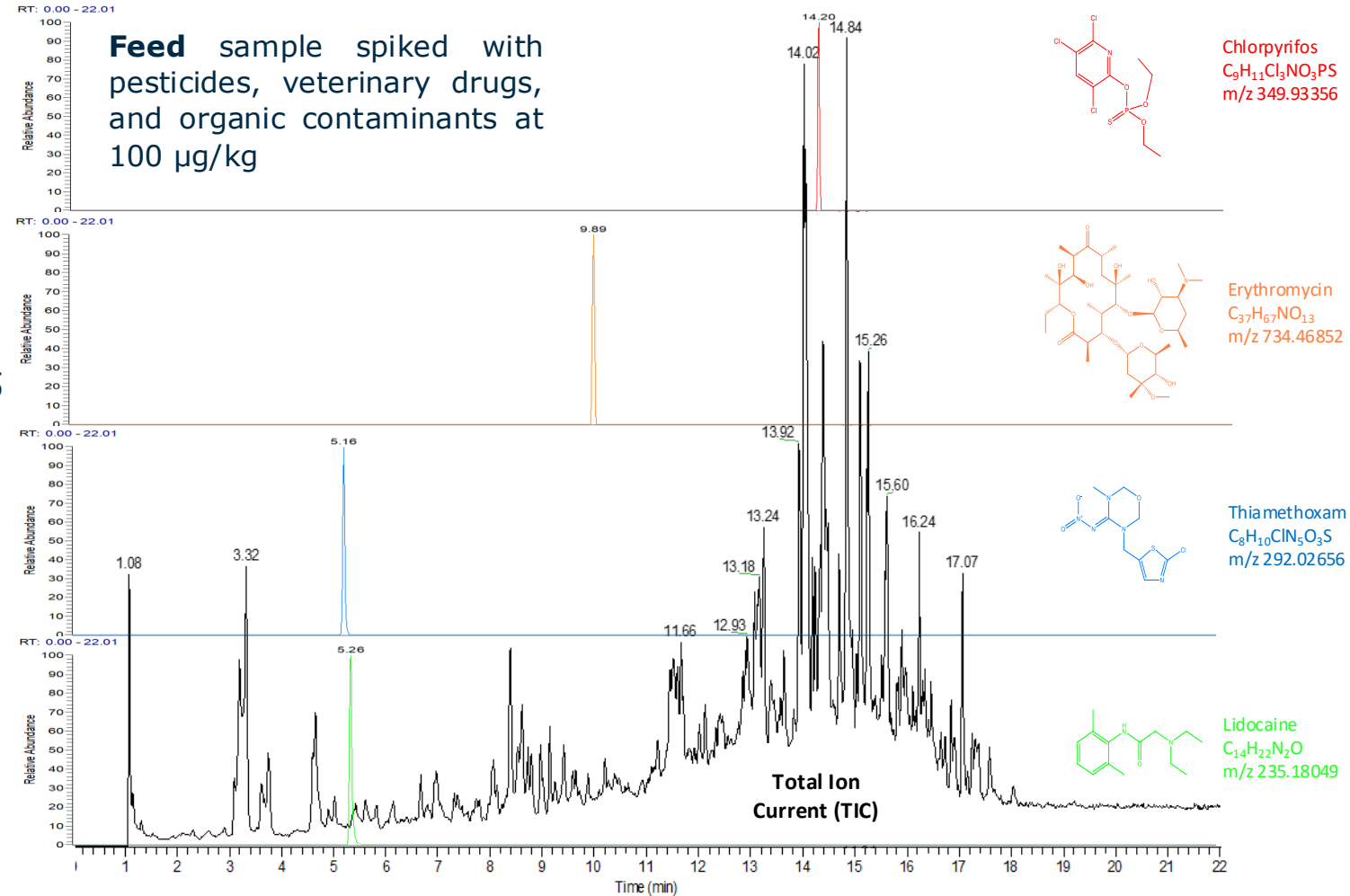
Extract signal of exact mass $\pm$ x Da (ppm) from the full scan



Chlorpyrifos => $C_9H_{11}Cl_3NO_3PS$ => 348.9262845

EIC = $[M+H]^+ = 349.93356 \pm 5$ ppm (± 0.00175 Da)

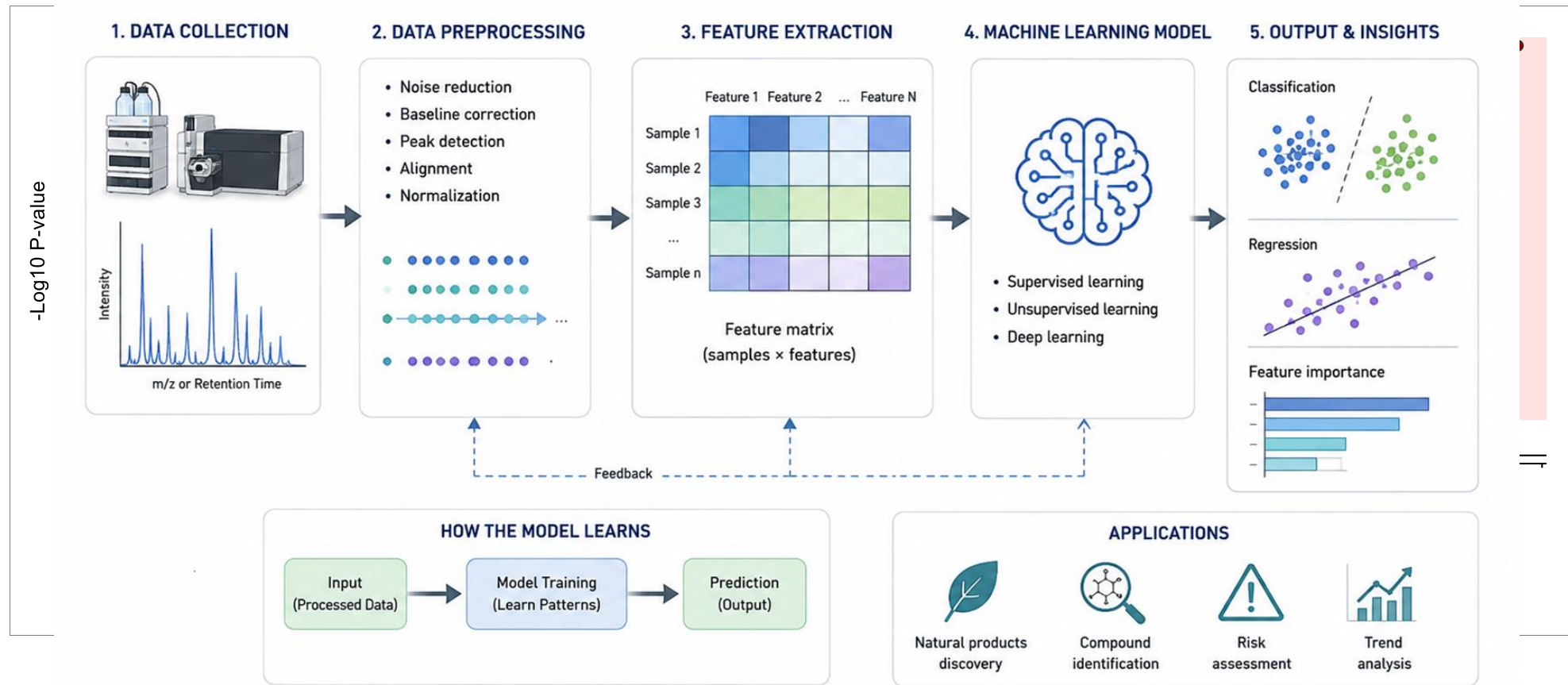
- **Target screening:** detect expected pesticides
- **Suspect screening:** detect extra pesticides
- **Retrospective analysis:** reanalyzing existing data for other compounds



The power of HRMS

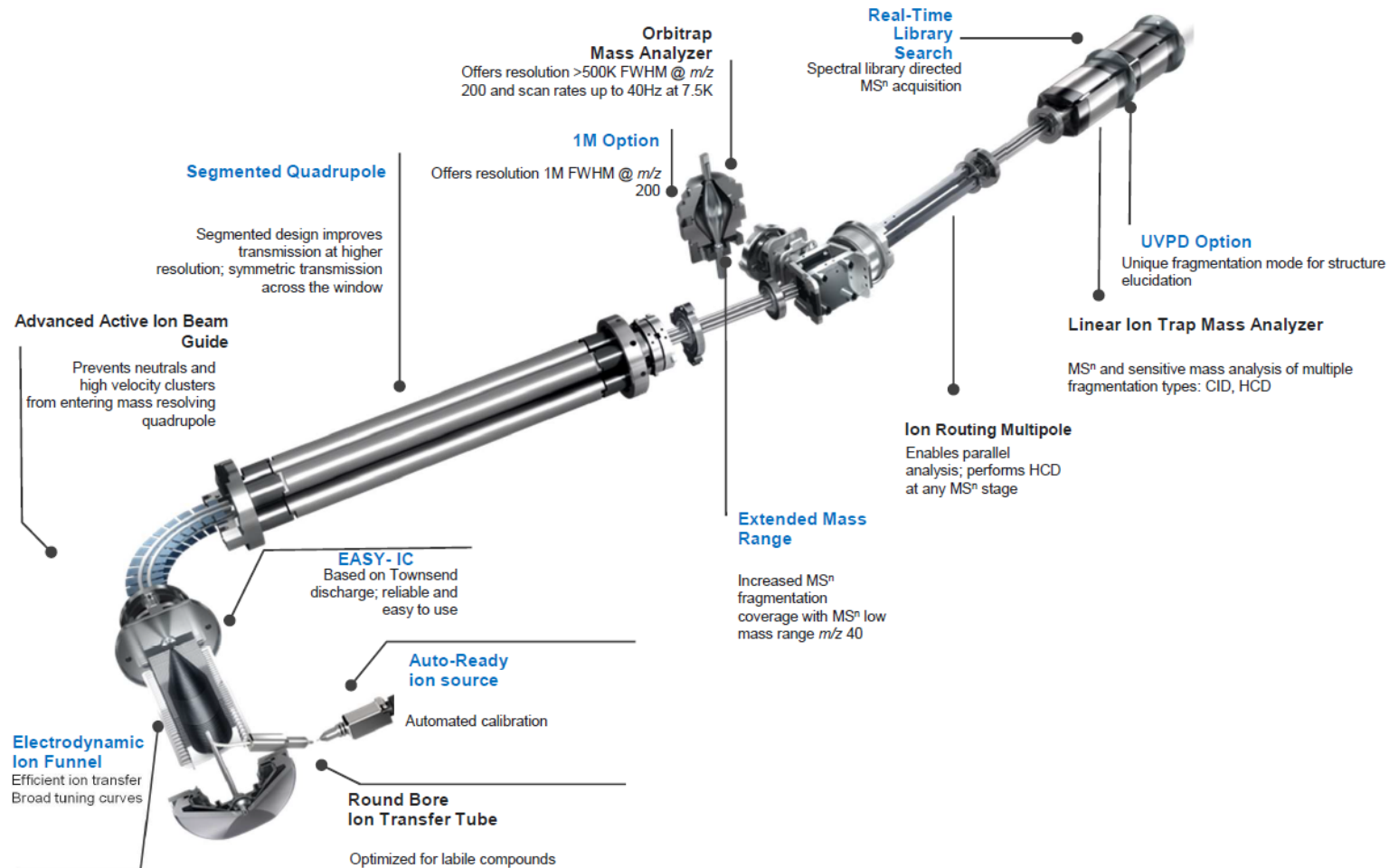
Untargeted screening: mass spectral libraries, molecular networking, differential analysis, and ML-based tools

Mass spectral libraries: databases containing reference mass spectra used to compare experimental spectra and help identify unknown compounds. Several open access options: **WFSR- Food Safety Spectral Library**, **MassBank**, **MoNA**, **GNPS**...
Machine learning based tools: computational approaches that group samples according to similar patterns from data sets based on their mass spectrum, classification, prediction, hypothesis, for **Structure Elongation**, **Compound Query**, **Compound Discoverer**, **GNPS**...



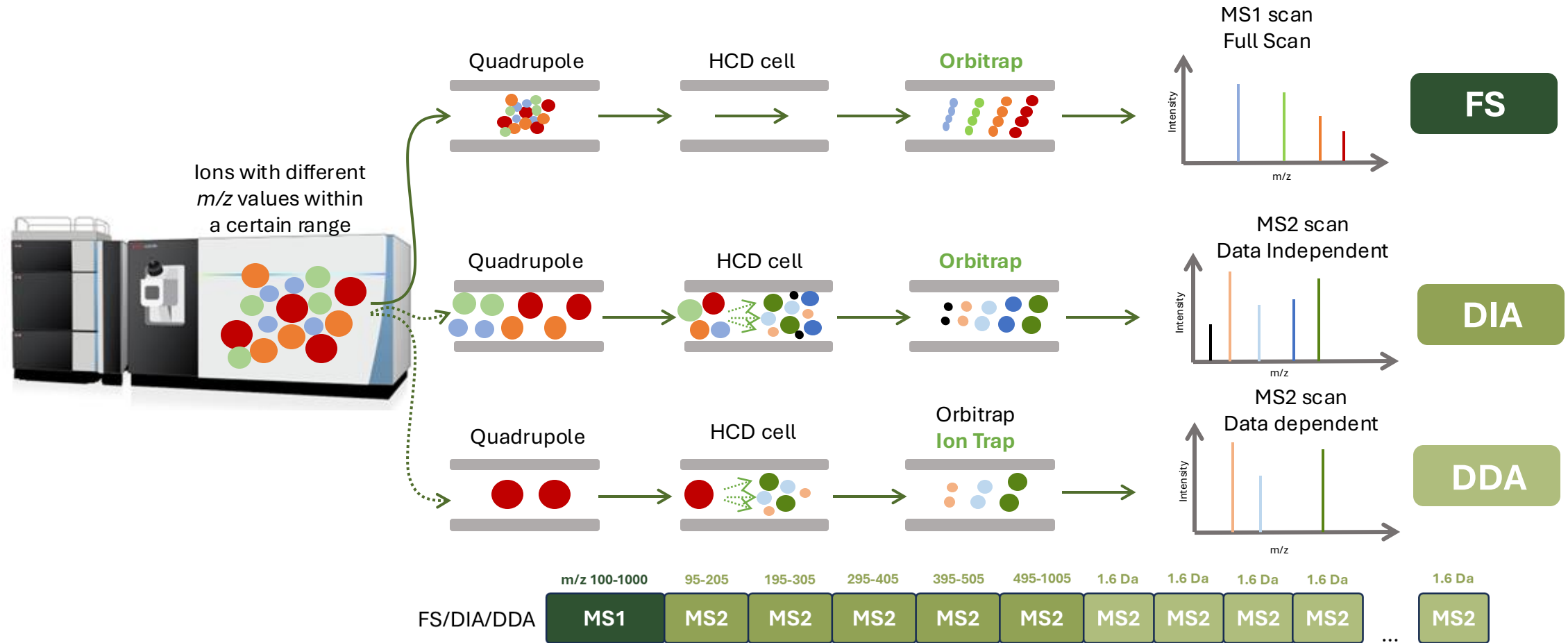
LC-HRMS

Inside the Orbitrap IQ-X mass spectrometer



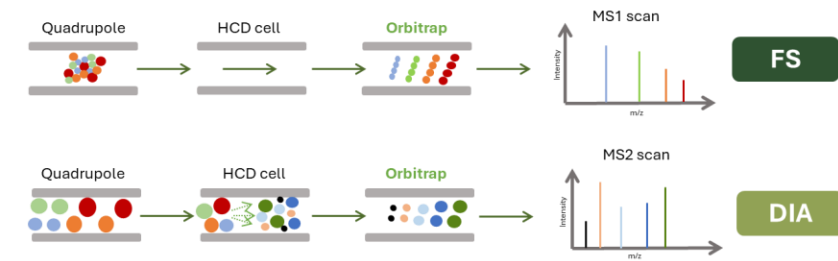
LC-HRMS

Data acquisition options

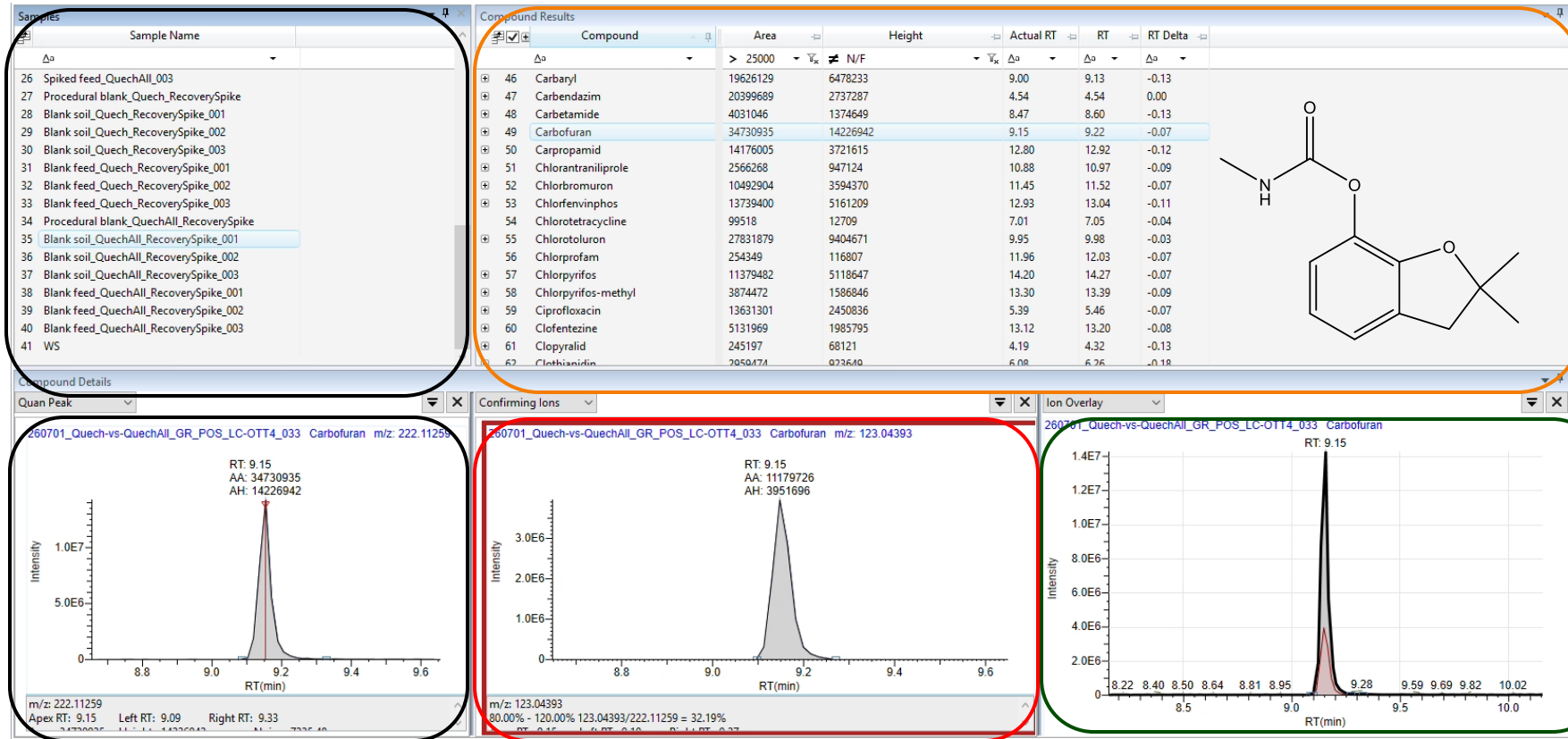


Data processing

DIA: The "2 ions approach"



Data files



Findings

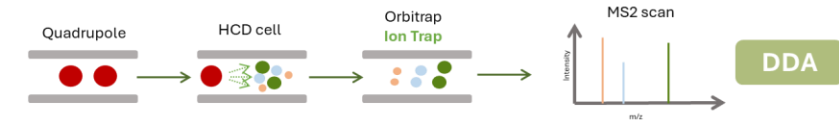
**EIC from the FS
Quantifier**

**Confirming ion from DIA
Qualifier**

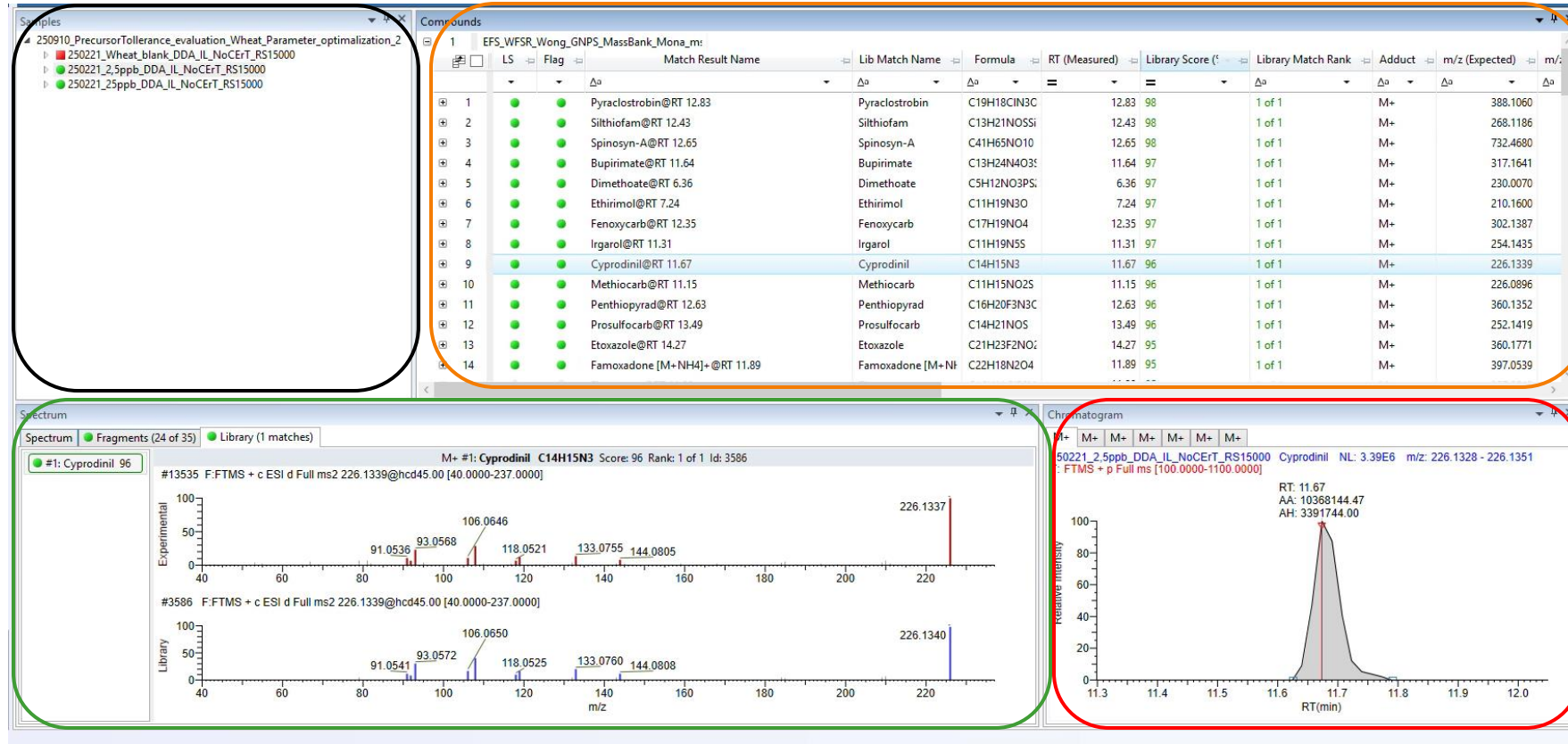
**Ions overlay
EIC + DIA**

Data processing

DDA: The mass spectral library match



Data files



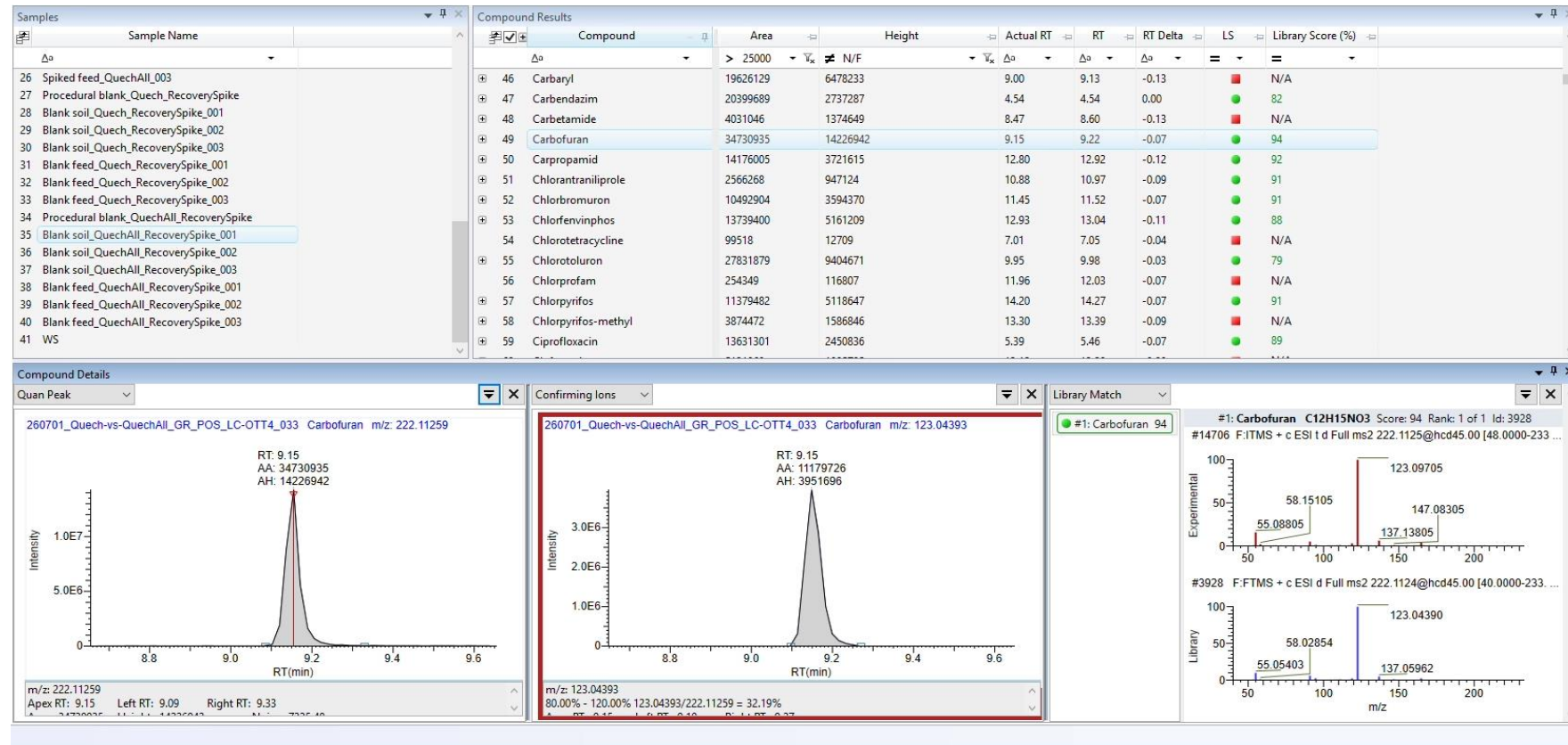
Findings

Extracted ion
chromatogram
EIC

Spectrum
Details about *FS* spectrum, fragments from
the database and *library* matches

Data processing

Combined: 2 ions approach + mass spectral library match



FS

DIA

DDA

Case study:

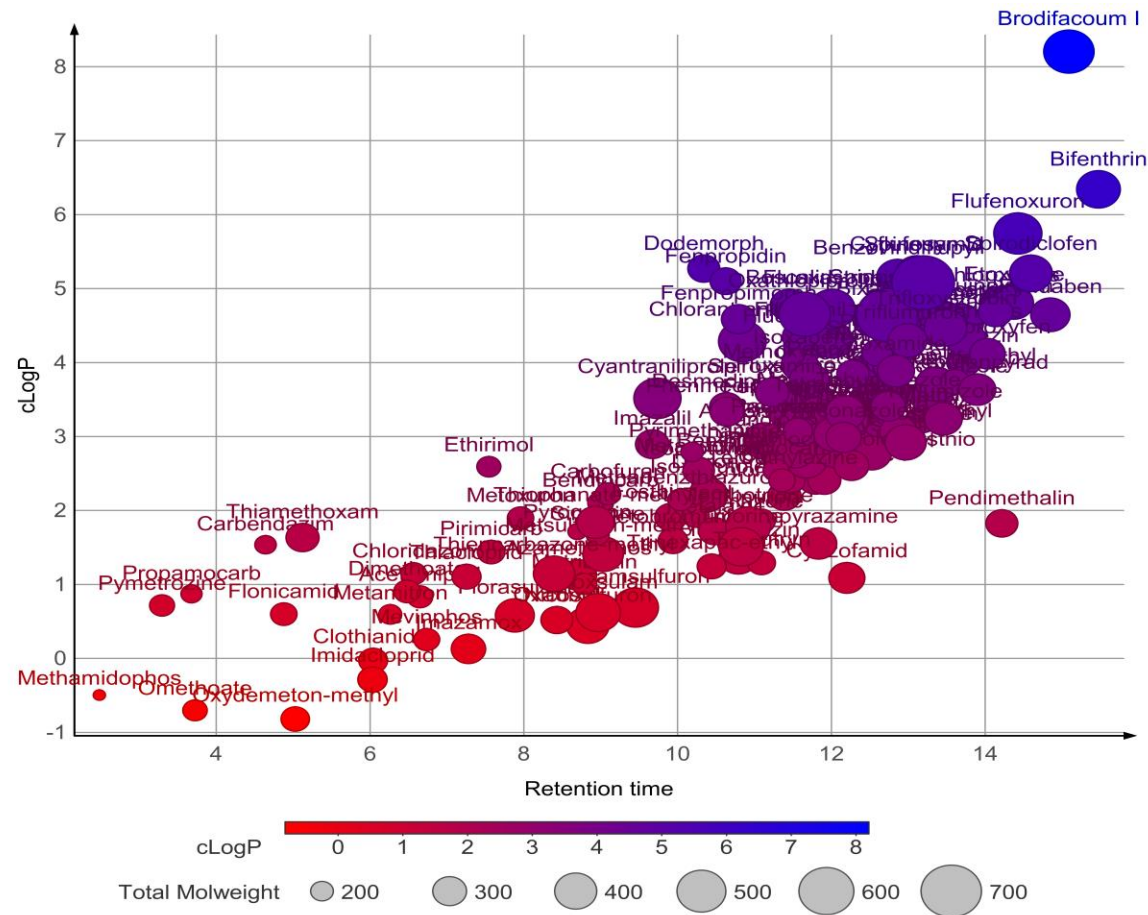
An Integrated LC-HRMS Strategy for Targeted and Untargeted Analysis of Food Toxicants

- Aim: development of a suspect screening method for the analysis of potential food toxicants in cereal-based processed food
- Possible food toxicants: pesticides, natural toxins, veterinary drugs, organic contaminants
- Different combination of detectors and acquisition strategies were tested using a mix of pesticides
- The final method combine targeted, suspect, and non-targeted screenings in a single data acquisition
- 100 cereal-based processed food were analyzed with the above mentioned method (bread, pasta, rice, breakfast cereals...)

Method optimization:

Analytes of interest

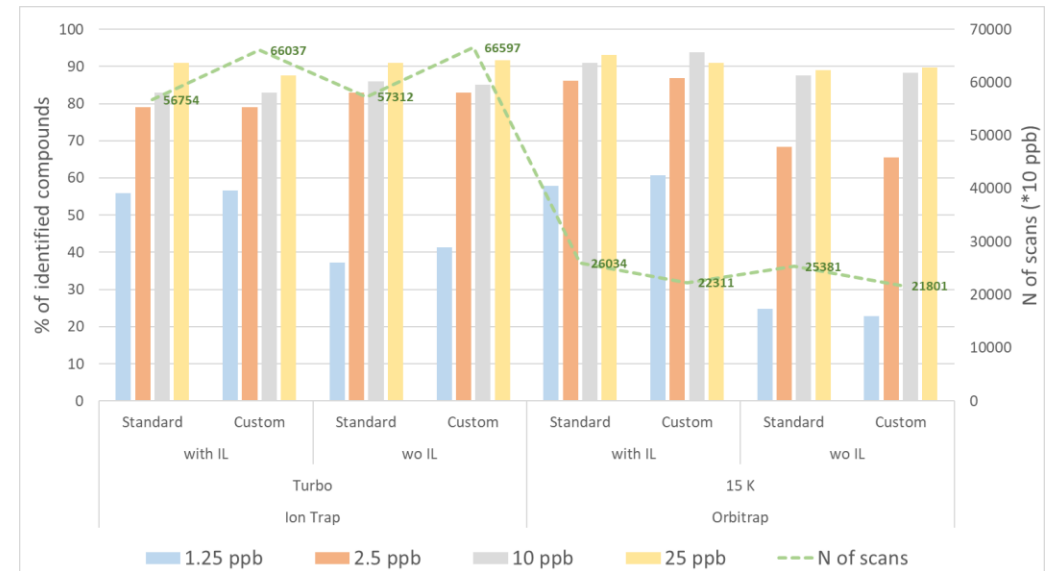
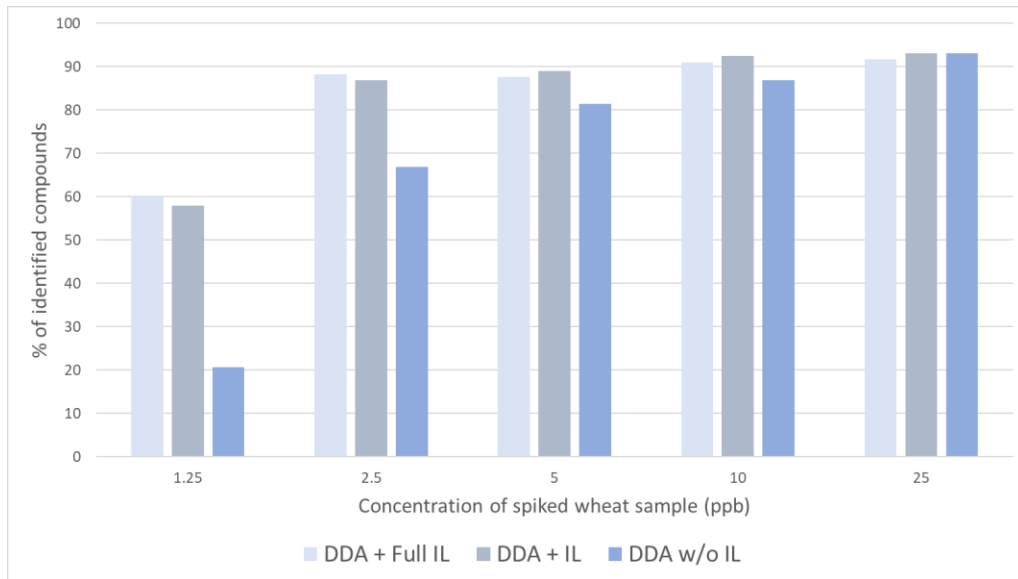
- Wheat was extracted using ACN (QuEChERS) and spiked with a mix of 145 pesticides



Method optimization:

Influence of different DDA triggering and MS2 detector

- A spiked wheat extract was used to evaluate which DDA strategy would result in the highest number of identified compounds



- The use of an inclusion list significantly improves triggering and identification, while retention times and specific ionization energies provide no additional benefit
- The number of DDA spectra acquired with Iontrap is 3× higher than Orbitrap

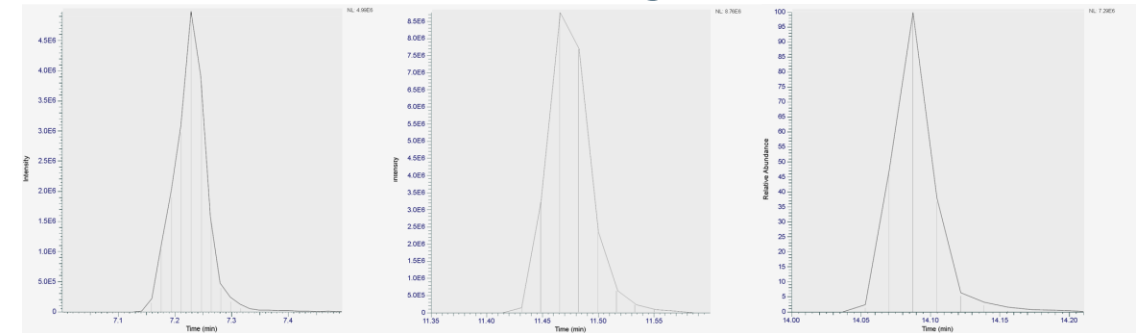
Method optimization:

Combining DDA and vDIA for MS2 data acquisition

- In the following experiments, the impact of combining vDIA and DDA within a single acquisition run was evaluated in terms of identified compounds and number of datapoints



[c]: 12.5 ng mL⁻¹



Thiachloprid

Paclobutrazol

Chlorpyrifos

- DIA with 2-ions/rt approach provides higher identification rates than DDA but requires the analytical reference standard
- DDA enables increased identification of compounds with unknown retention times but require the presence of the spectrum in a spectral library

Method optimization:

Selected experimental conditions



Column: Waters BEH C18 column (2.1 mm×100 mm, 1.7 µm particle size)

Flow rate and injection volume: 300 µL min⁻¹; 5 µL

Mobile phases: (A) H₂O + 2 mM NH₄COOH and 0.1% HCOOH

(B) MeOH + 2 mM NH₄COOH and 0.1% HCOOH

Gradient: from 0% B (0 min) to 100% B (15 min) – Elution phase

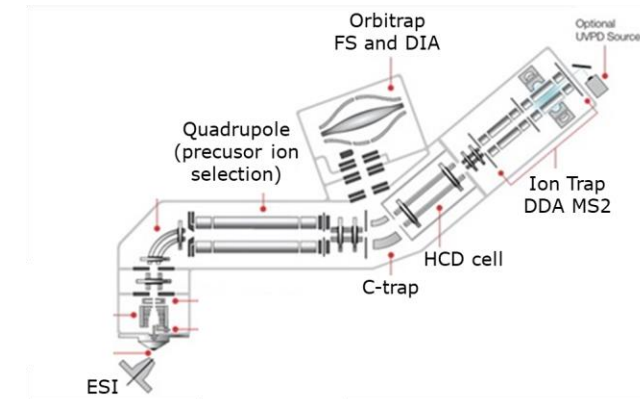
100% B (15-21 min)– Washing phase

from 100% B (21 min) to 0% B (22-25 min) – conditioning phase

Full Scan (FS): Orbitrap; 100–1100 *m/z*; 60,000 fwhm (*m/z* 200)

Data Dependent (DDA): Ion Trap; Collision Energy (CE) 45%

Data Independent (vDIA): Orbitrap; 30,000 fwhm (*m/z* 200); CE 30,80% (avr.)

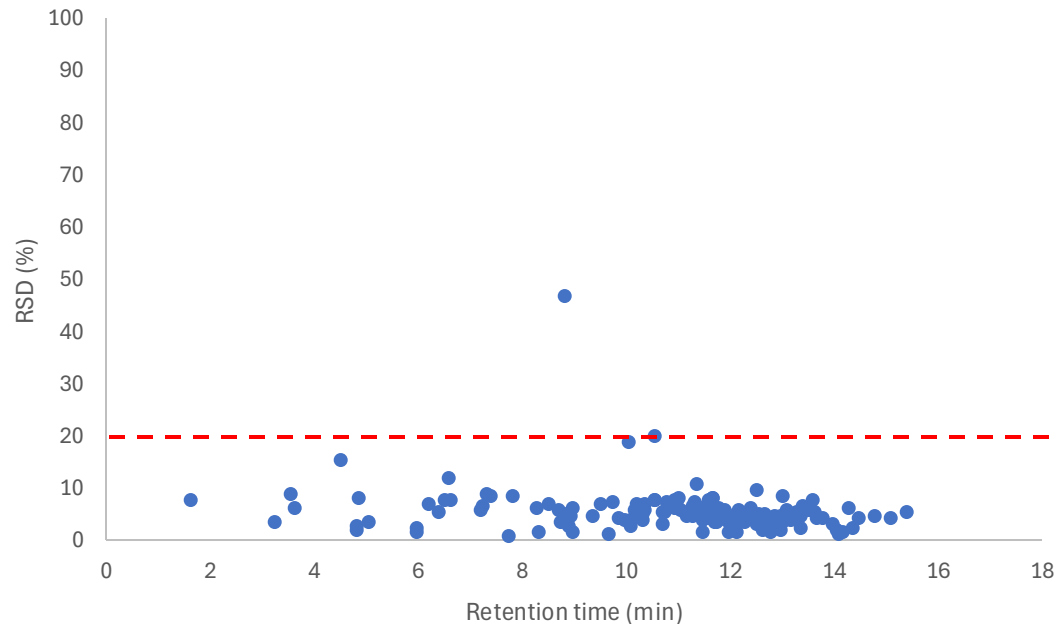


Method optimization:

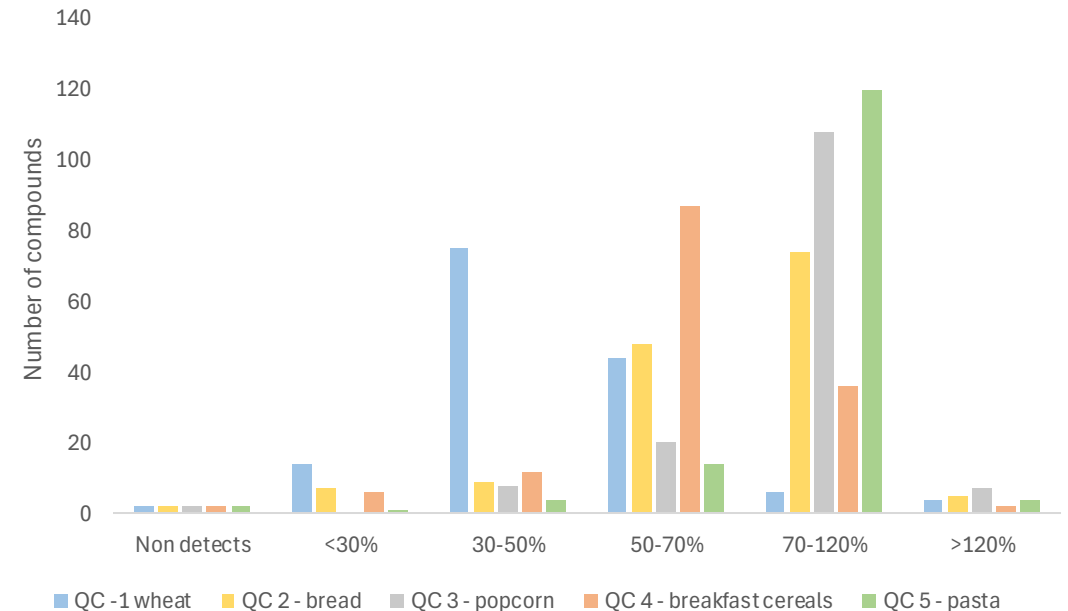
Method performance

- System stability was evaluated on the 145 pesticides in solvent at 12.5 ng/mL (n = 6)
- Apparent recovery was calculated on the 145 pesticides spiked at 50 µg/kg, prior extraction

System stability



Apparent recovery



Results

Findings

- 72 food toxicants were detected in 100 cereal products

Pesticides

Pirimiphos methyl	70
Tebuconazole	68
Piperonyl butoxide	51
Azoxystrobin	37
Hydroxy-tebuconazole	31
Famoxadone	11
Fluxapyroxad	17
Fluopyram	11
Benzovindiflupyr	4
Tebuconazole	4
Ametoctradin	3
Epoxiconazole	3
Imidacloprid	3
Tetraconazole	3
Chlorpyrifos	2
Cyproconazole	2
Spinosyn D	2
3-Hydroxycarbofuran	1
Acetamiprid	1
Bixafen	1
Boscalid	1
Difenoconazole	1
Fluopicolide	1
Imazapic	1
Pirimicarb	1
Propamocarb	1
Propanil	1
Quinlorac	1
Sedaxane	1
Spirotetramat-enol	1
Spirotetramat-mono-hydroxy	1
Trifloxystrobin	1
8-Hydroxyquinoline	9
Flubendazole	1
Matrine	11

Natural toxins

Enniatin A1	74
Enniatin B	74
Ergocristinine	42 45
Ergocristine	42 31
Beauvericin	41
α -Ergocryptine	28 17
α -Ergocryptinine	28
β -Ergocryptine	28 17
Deltamethrin	16
Sophoridine; Allomatrine	9
Isolupanine	11 14
Lupanine	11 14
Sterigmatocystin	5 22
γ -Chaconine	5 2
Androstanolone	4
Spinosyn-A	3
Oxysophocarpine	2
Solasodine	2
Aflatoxin B1	1
Enniatin B1	1
Coumarin	4
Demissidine; Sotamin D	4
Enniatin A	17
Piperine	13
Thebaine	1
Tetrahydrocannabinol (THC)	1

Veterinary drugs

19-Nor-epi-androsterone	66
5α-Estrane-3,17-dione	62
Nandrotone	44
19-Norandrosterone	48
Progesterone	9 59
Dehydroepiandrosterone	7
5α-Androstan-3,17-dione	4
Hydroxyflubendazol	2 1

Organic contaminants

2,4,6-Tri-tert-butylphenol	71
Cyclohexanone oxime	5

~~Compound~~ = false positives (9)

Compound = N/A (5)

Compound = identified (13)

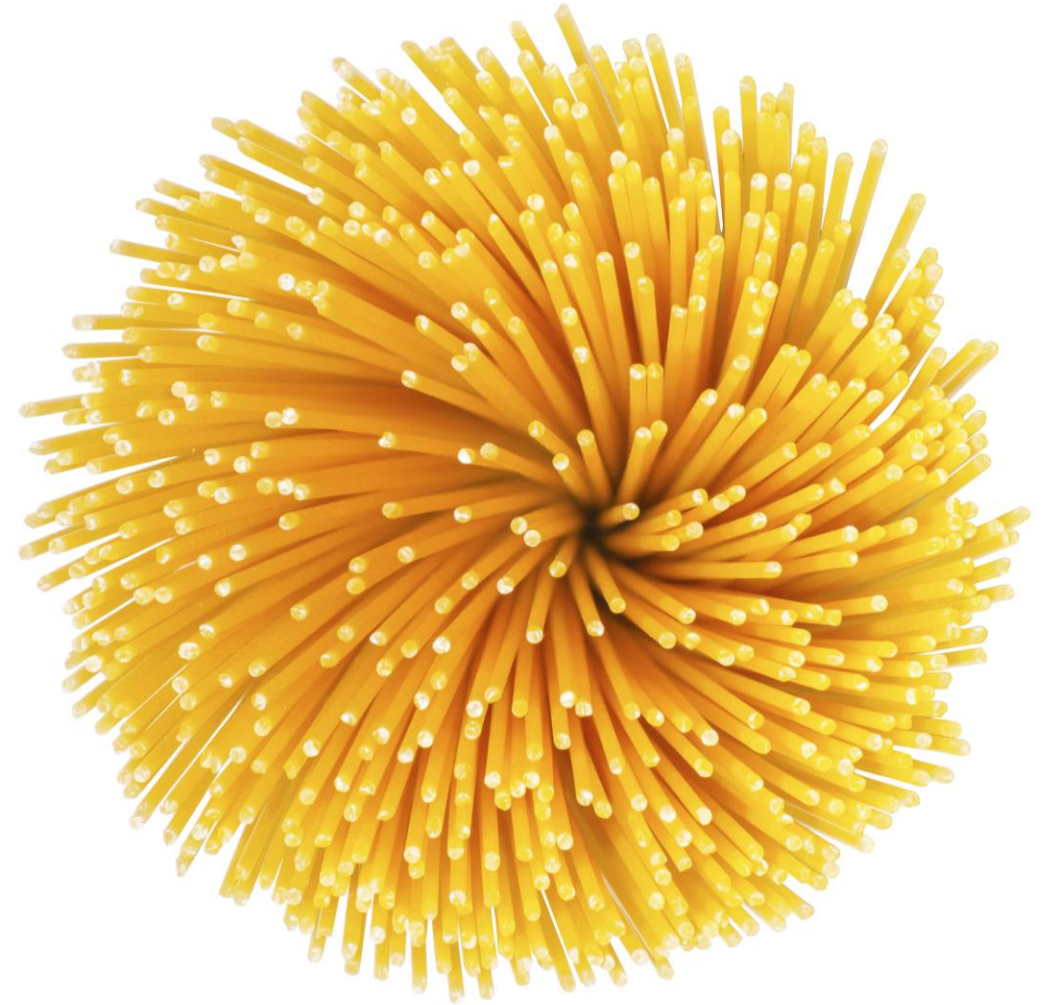
Compound = tentative (45)

Conclusions

- The combination of quantitative analysis and different screening strategies in a single measurement is possible without compromising the instrumental performances
- A suspect screening method for the analysis of potential food toxicants in cereal-based processed food was developed and applied on 100 samples
- DIA provides higher identification rates than DDA but requires known retention times for accurate compound identification.
- The optimized LC-HRMS method effectively detected a wide range of food toxicants in cereal-based processed products
- The finalized method is suitable for broad screening applications in food safety monitoring

Thank you for
your attention!

Questions?



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