FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

IAEA
International Atomic Energy Agency

SweEt multiresidue method

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Livsmedelsverket

RALACA
Red Analítica de Latinoamérica y el Caribe

Outline

- National Food Agency
- SweEt pros and cons
- SweEt workflow
- LC- and GC-MS/MS
- Proficiency Test
- Screening with HRMS

Uppsala to Foz Do Iguacu

The National Food Agency

We work for:

- safe food and drinking water
- fair practices in the food trade
- healthy eating habits

Director General

Advisory Council

Science Division

- Biology
- Chemistry
- Risk Benefit Assessment
- Advice and Emergency preparedness

Food Control Division

- Analysis
- Planning
- Support
- Northern and Central Sweden
- Western Götaland
- Eastern Götaland
- Southern Götaland

Strategic Development and Support Division

- Finance and Accounting
- Human Resources
- IT and Service
- International Affairs
- Legal Affairs
- Communications
- Evaluation

Livsmedelsverket

"Nothing is more simple than greatness; indeed, to be simple is to be great."

514

Extraction

10 g sample
20 ml EtOAc+$NaHCO_3$
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GC-MS/MS

UHPLC-MS/MS + HRMS

359

Extraction

5 g sample
10 ml water
10 ml EtOAc (1% HAc)
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GC-MS/MS

UHPLC-MS/MS

119

Extraction

5 g sample
PSA + C18
10 ml EtOAc
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GC-MS/MS

UHPLC-MS/MS

78

Fat determination

Extraction

0.5 g fat
10 ml EtOAc:CH (1+1)
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GPC SX-3
(1+1 EtOAc/CH)

Evaporation

Reconstitution in EtOAc

GC-MS/MS

UHPLC-MS/MS

SweEt multiresidue method – a NMKL method and an ongoing CEN-method

SweEt - pros and cons

+ Fast
+ Cost effective
+ Environmentally friendly
+ Extracts can be analyzed directly on GC- and LC-MS/MS
+ Matrix components as proteins and sugar are extracted at very low levels

- Analyses of EtAc (or ACN with QuEChERS) on LC-MS/MS where water buffer and methanol are used as mobile phase, solvent effects arise due to that the solubility between sample extract and mobile phase is limited.

The study of different extraction solvents and the amount of co-extracted matrix components

			Type of solvent				
Matrix	**Matrix type**	**Amount of extracted sample**	**Acetone /water 2+1**	**Acetonitrile /water 2+1**	**Methanol /water 2+1**	**Water**	**Ethyl acetate**
Tomato	High water content	1.0g	38mg	36mg	34mg	33mg	8mg
Citrus	High acid and high water content	1.0g	79mg	69mg	81mg	-	3mg
Raisins	High sugar an low water content	1.0g	370mg	148mg	333mg	302mg	4mg
Avocado	High oil content	1.0g	33mg	40mg	30mg	43mg	**332mg**

Livsmedelsverket

J. Klein and L. Alder: Development of a Multi-Analyte / Multi-Matrix Method Based on LC-MS/MS; EPRW 2002, Rome 28.- 31.05.2002

Workflow for Fruits and Vegetables

Sample treatment – stainless steel gloves

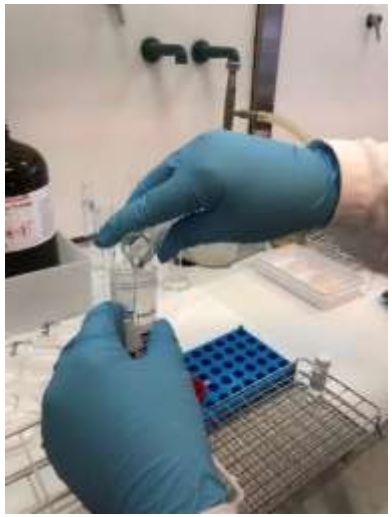
Livsmedelsverket

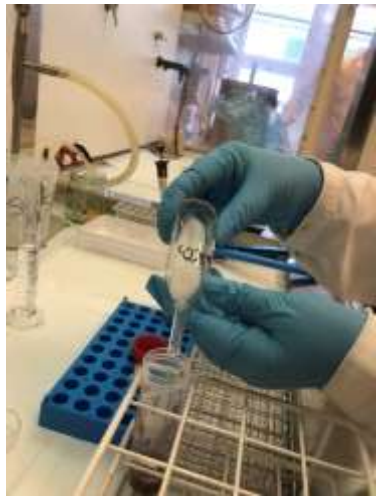
Homogenization

Cut-o-mat

Livsmedelsverket

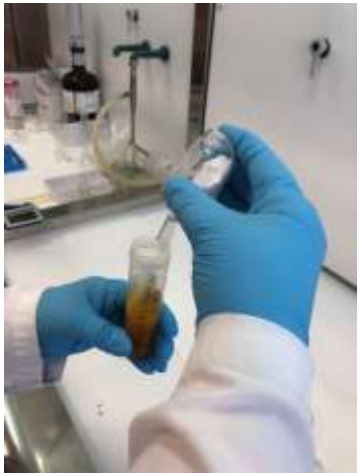
Sample – Ethyl acetate – Internal standard - $NaHCO_3$





Livsmedelsverket

Extraction – 3+1 min

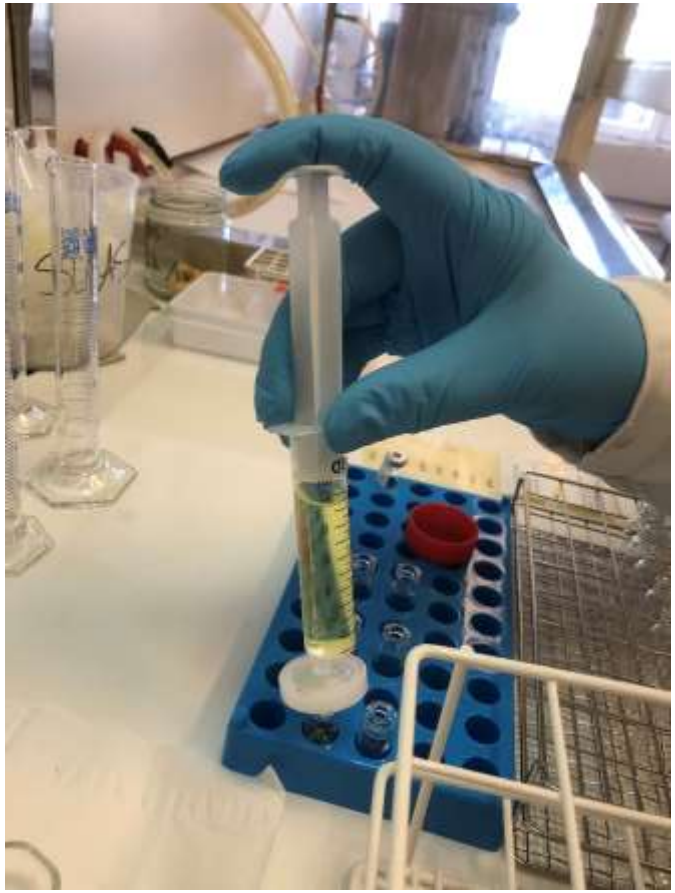


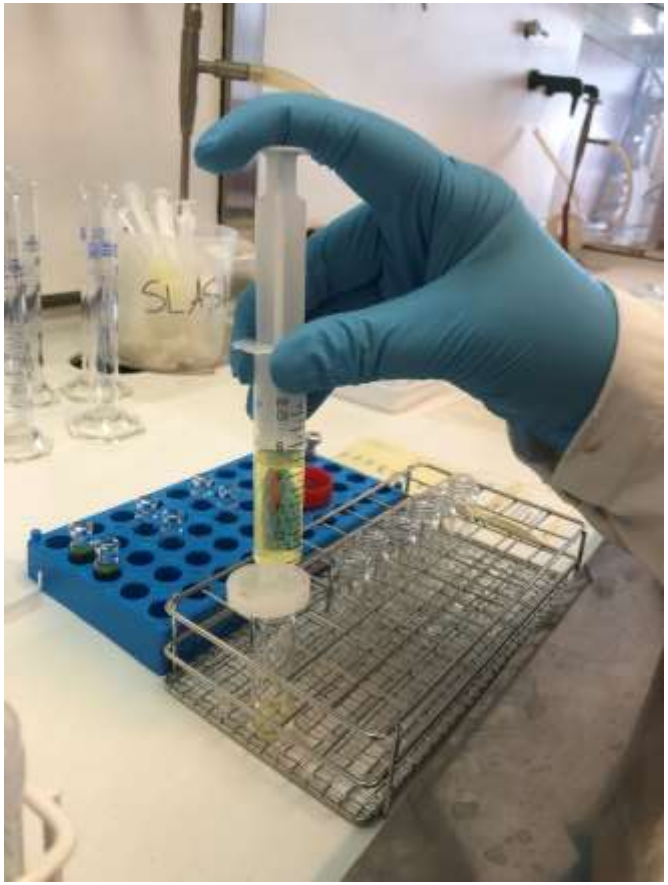
Livsmedelsverket

Centrifugation – 3 min at 3200 g

Livsmedelsverket

Filtration





Livsmedelsverket

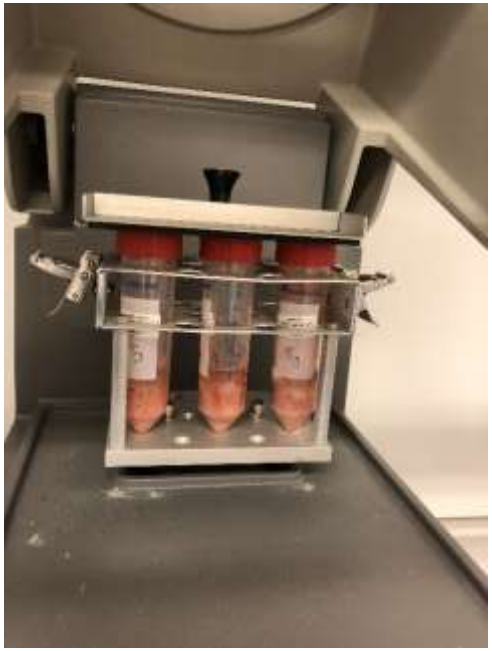
Ready for analyses on LC-MS/MS and GC-MS/MS

Sample treatment & homogenization: 10 min
(~1kg oranges chopped and homogenized)
Extraction: 3 min
Centrifugation: 3 min
Filtration: 1 min

Livsmedelsverket

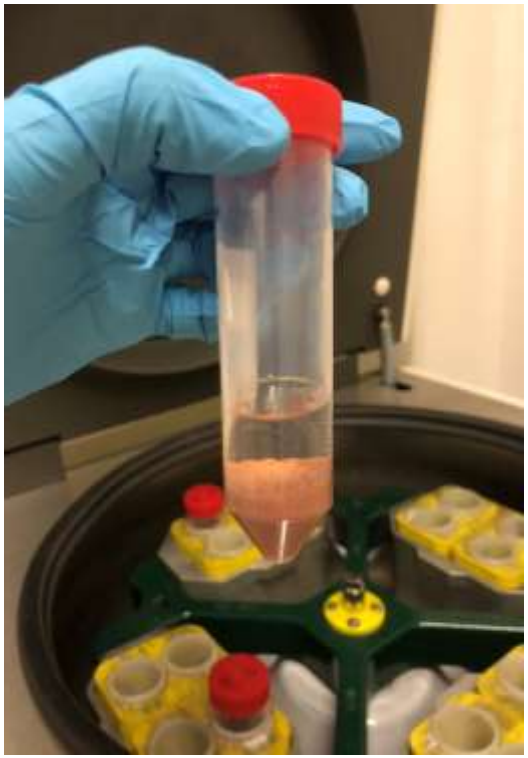
SweEt - Animal of origin

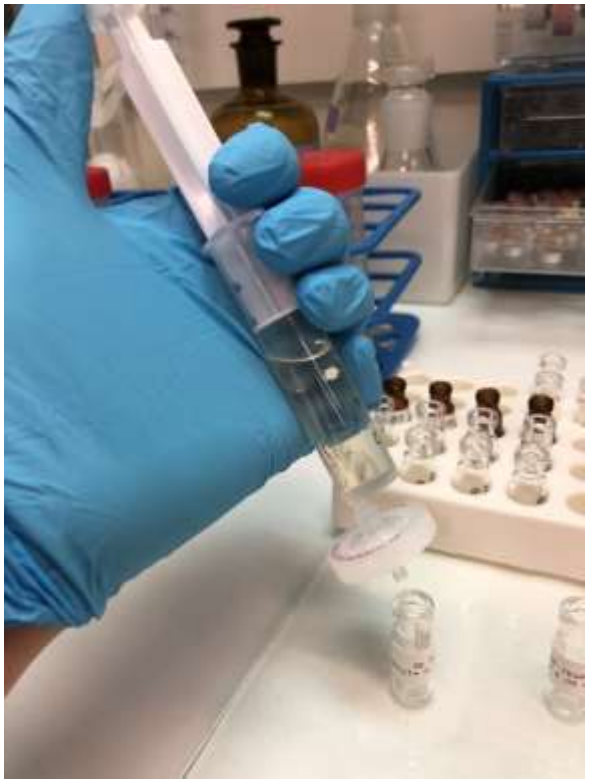
PSA and C18



Livsmedelsverket

SweEt-AO
Centrifugation and filtration





Livsmedelsverket

LC-MS/MS parameters

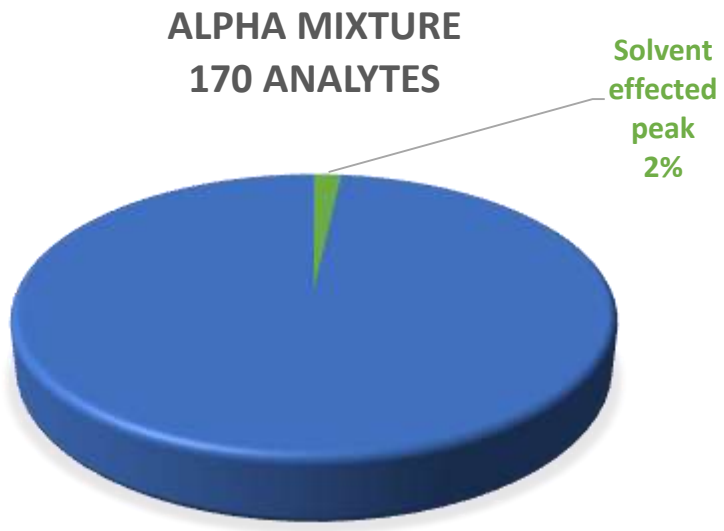
Total Time (min)	B (%)	A (%)
0	5	95
0,2	5	95
0.5	50	50
2.5	55	45
5.5	75	25
8	85	15
11	100	0
13	100	0
13.1	5	95
16	5	95

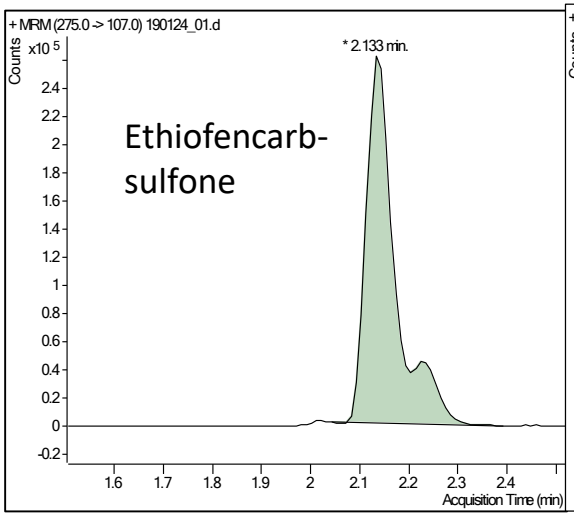
For analyses with UPLC-MS/MS (ES+/ES-)

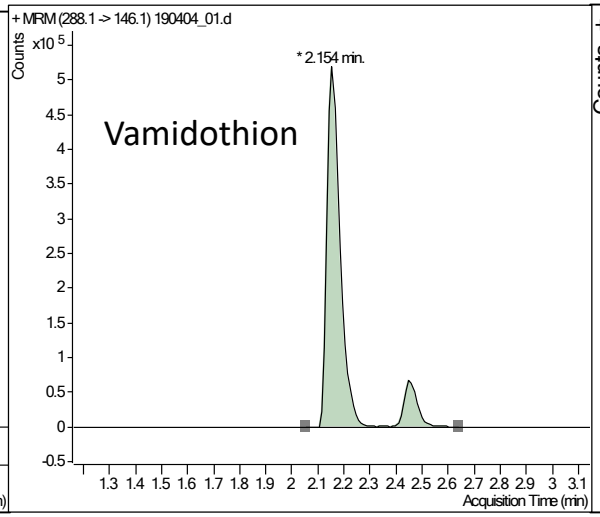
Column temp: 45 °C
Pre column: Waters Pre-Column HSS T3, 5 x 2.1 mm, 1.8 µm
Column: Waters HSS T3, 150 x 2.1mm, 1.8 µm
Mobile phase A: 10 mM ammonium formiat, pH 4
Mobile phase B: Methanol
Flow rate: 0.45 µl/min
Inj vol: 2-5 µl

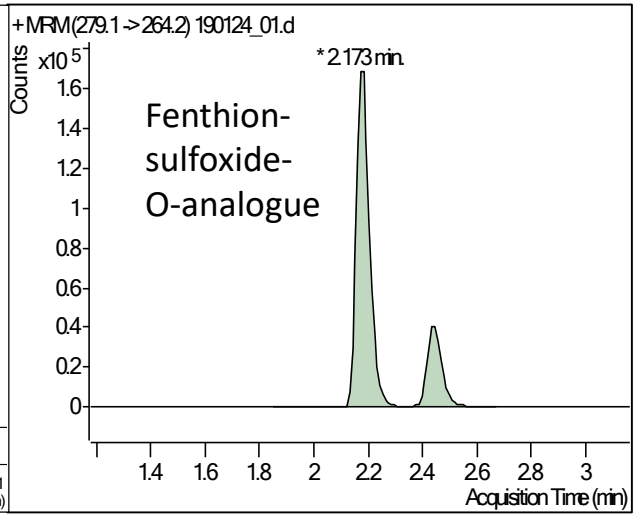
Livsmedelsverket

Solvent effected peaks

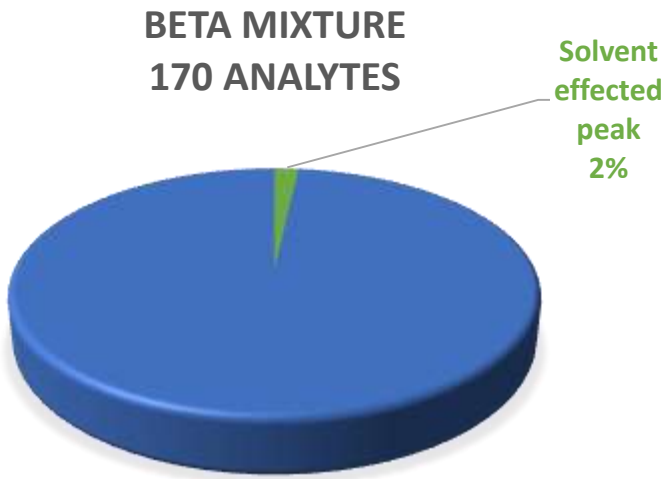


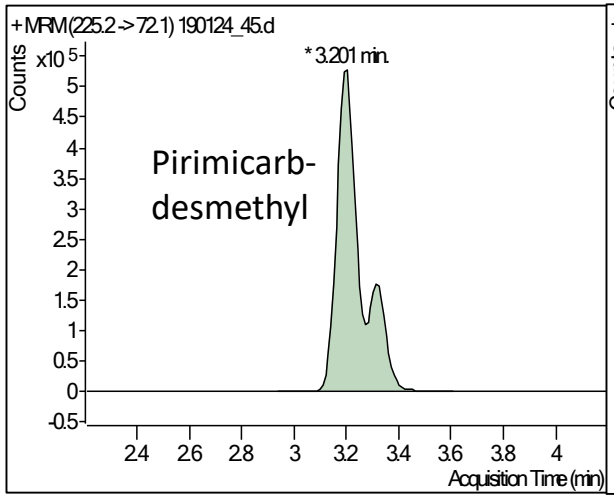


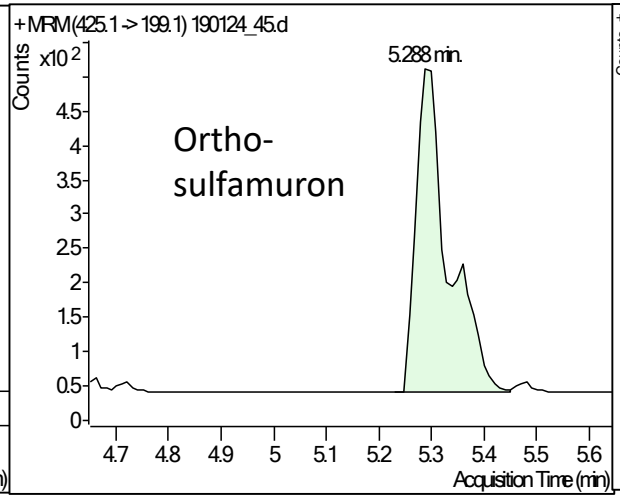


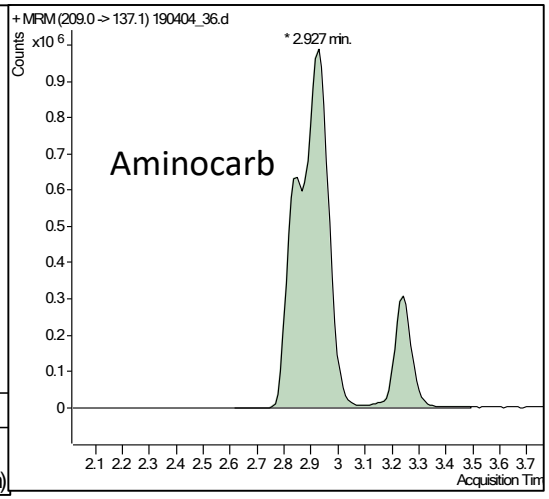


Solvent effected peaks



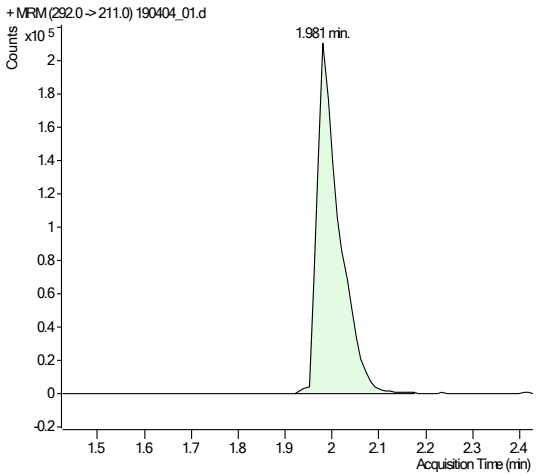




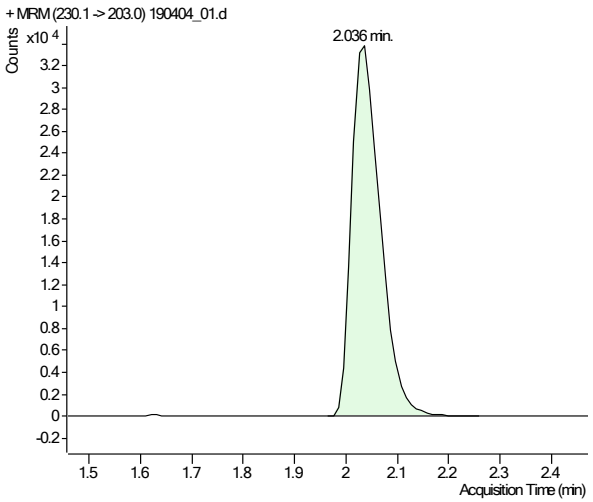


Examples of early eluting analytes around 2 minutes

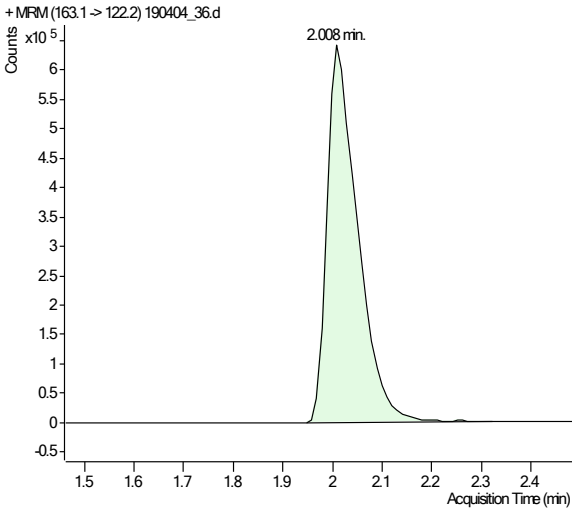
Thiamethoxam



Flonicamid



DMPF



New improved gradient gives less peaks with solvent effects (due to direct injection with in ethyl acetate).

Livsmedelsverket

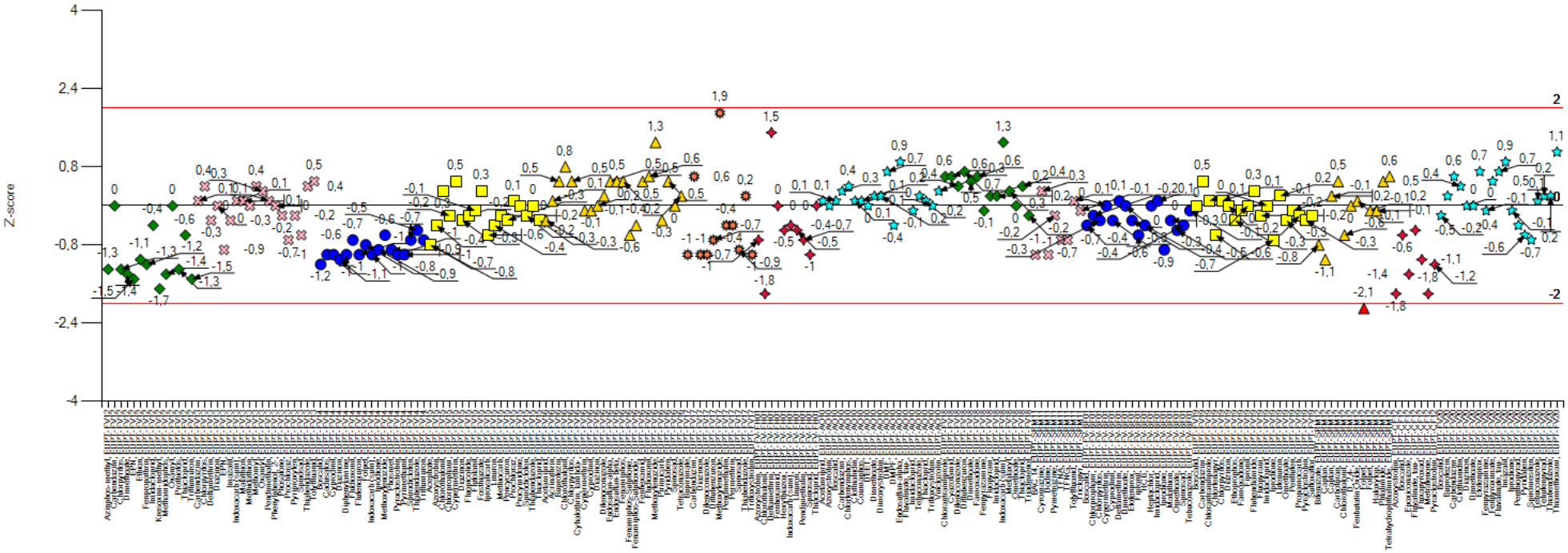
GC-MS/MS parameters

Injection technique	Large volume MMI with post-run, mid-column backflush. Pulsed splitless
Carrier gas	Helium
Liner	Dimpled, splitless, Ultra Inert Liner
Inj temp programme	Initial 70 °C, Initial time 0.1 min, Temp gradient 600 °C/min, End 325 °C
Inj Pulse Pressure	30 psi until 0.5 min
Splitless time	0.75 min
Injection	1 µl
Column	2 x HP-5MS Ultra Inert 15 m x 250 µm x 0.25 µm, 19091S-431UI
Gas flow	1 ml/min (constant flow)
Collision gas	Nitrogen
Solvent Delay	4.5 min
Transfer line	280 °C
Ion source	High Efficiency Source, EI 250 °C
Oven, init temp	70 °C
Initial time	1.0 min

Oven temperature programme

Gradient (°C/min)	Final temperature (°C)	Isothermal (min)
30	200	0.00
5	280	3.00
30	320	1.00

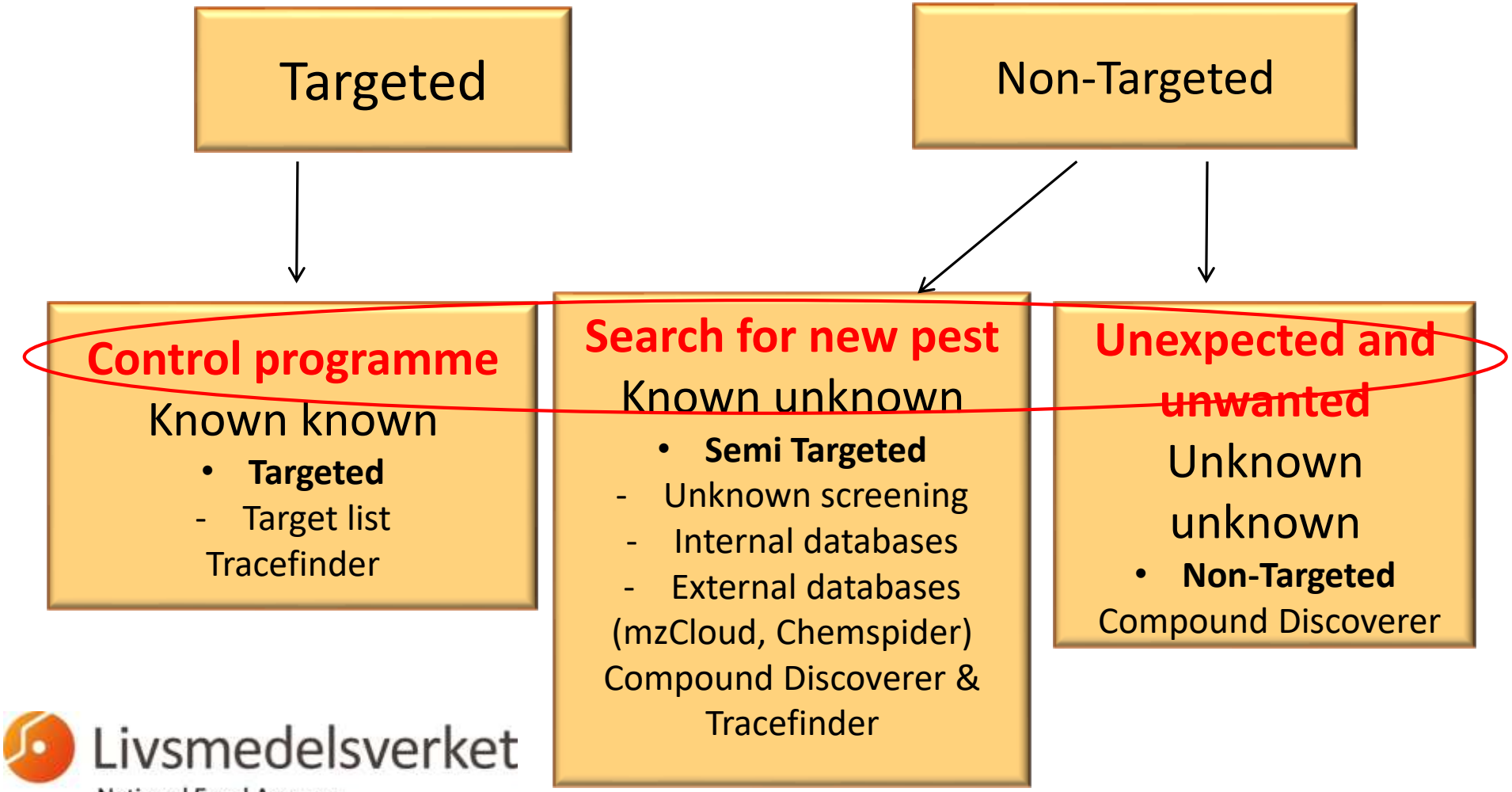
EUPT – SweEt results



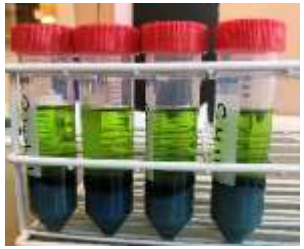
- EUPT-FV12 Leek
- EUPT-FV13 Mandarin
- EUPT-FV14 Pear
- EUPT-FV15 Potato
- EUPT-FV16 Pepper
- EUPT-FV17 Broccoli
- EUPT-FV-FH01 Coriander
- EUPT-AO10 Honey
- EUPT-FV18 Spinach
- EUPT-SRM11 Spinach
- EUPT-FV-BF01 Peach
- EUPT-FV19 Lemon
- EUPT-SRM12 Strawberry
- EUPT—CF12 Hay
- EUPT-FV20 Bean
- EUPT-FV21 Red cabbage

Livsmedelsverket
National Food Agency

Workflow for Targeted and Non-Targeted Analyses



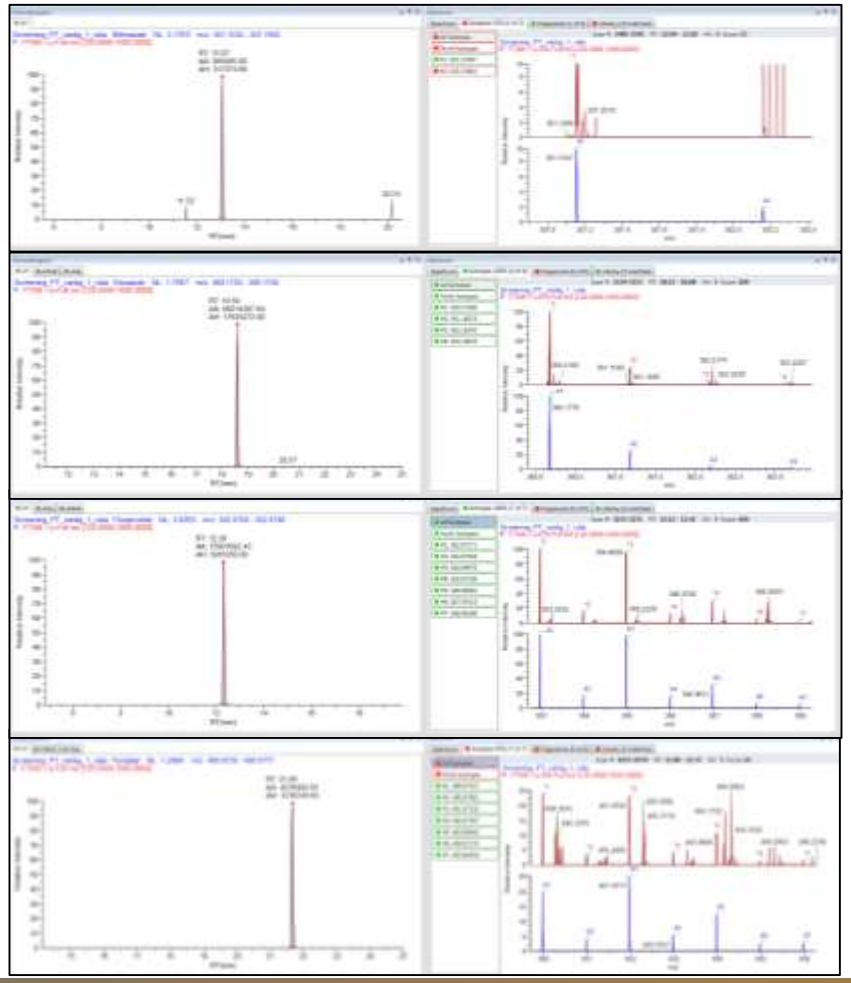
Livsmedelsverket
National Food Agency



SM11 - EUPT Screening in F&V 2019 red cabbage with Q Exactive Orbitrap

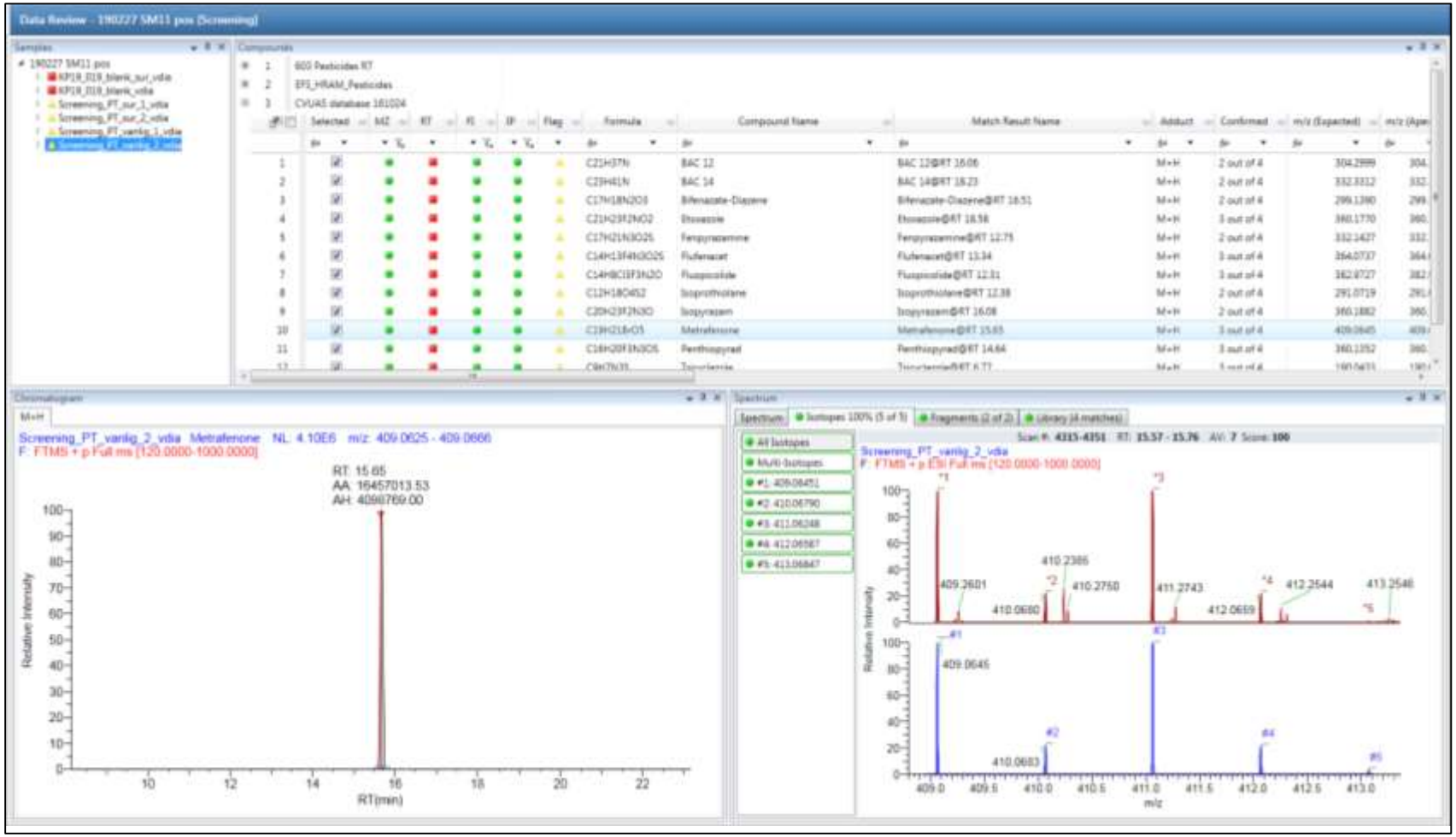
NFA, 1 of 5 out of 69 laboratories to score 100% (17 analytes).

Bifenazate-GC
Bifenazate diazene
Etoxazole-GC
Fenpyrazamine
Flubendiamide
Flufenacet
Fluopicolide-GC
Isoprothiolane
Isopyrazam
Metrafenone
Orthosulfamuron
Penthiopyrad
Propoxur
Pyridalil-GC
Spinetoram
Tricyclazole
Valifenalate



Preliminary results

EUPT SM11 on LC-MS Orbitrap
Metrafenone



Livsmedelsverket
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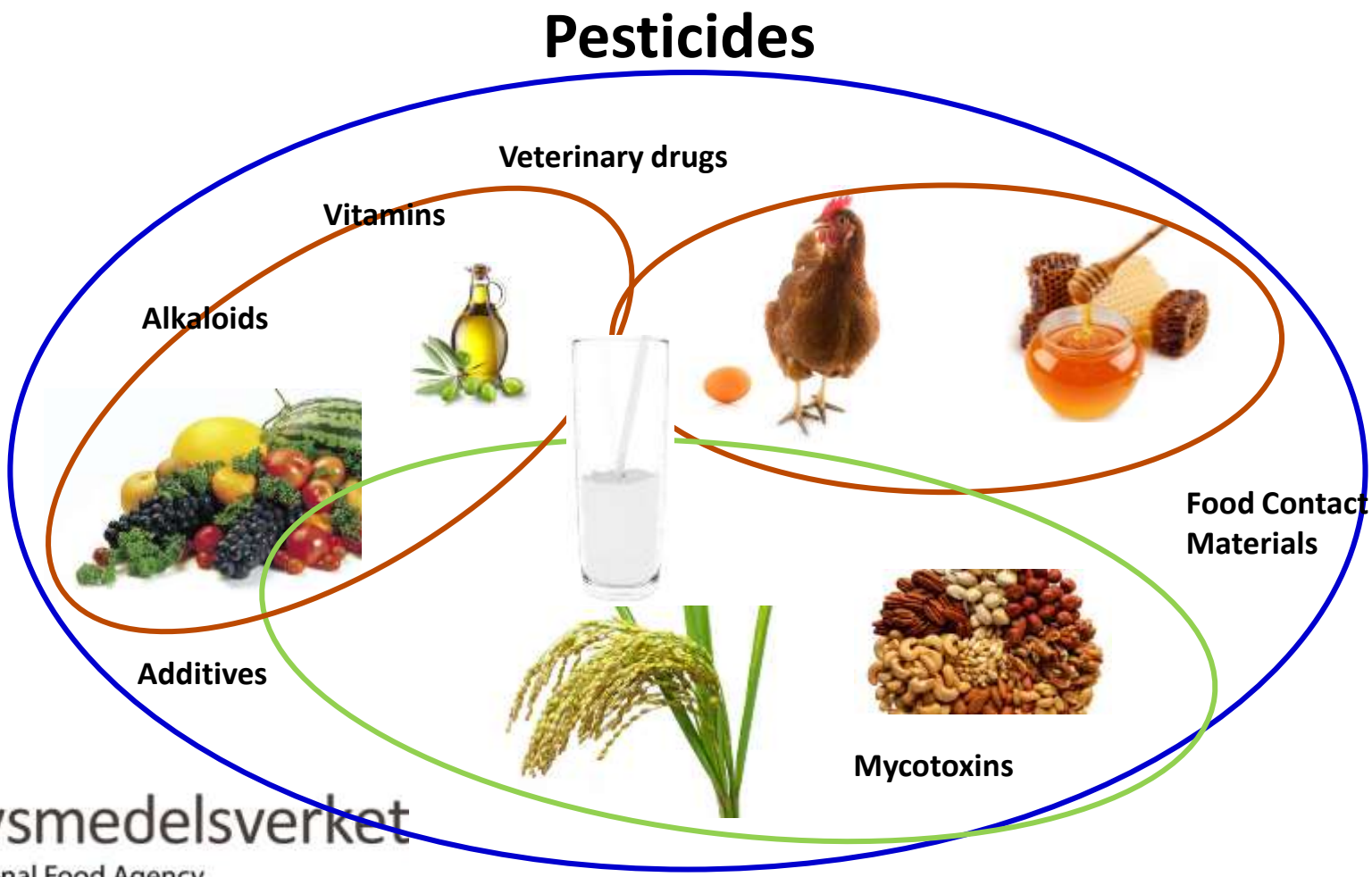
Screen for several different compound classes in same sample



Livsmedelsverket
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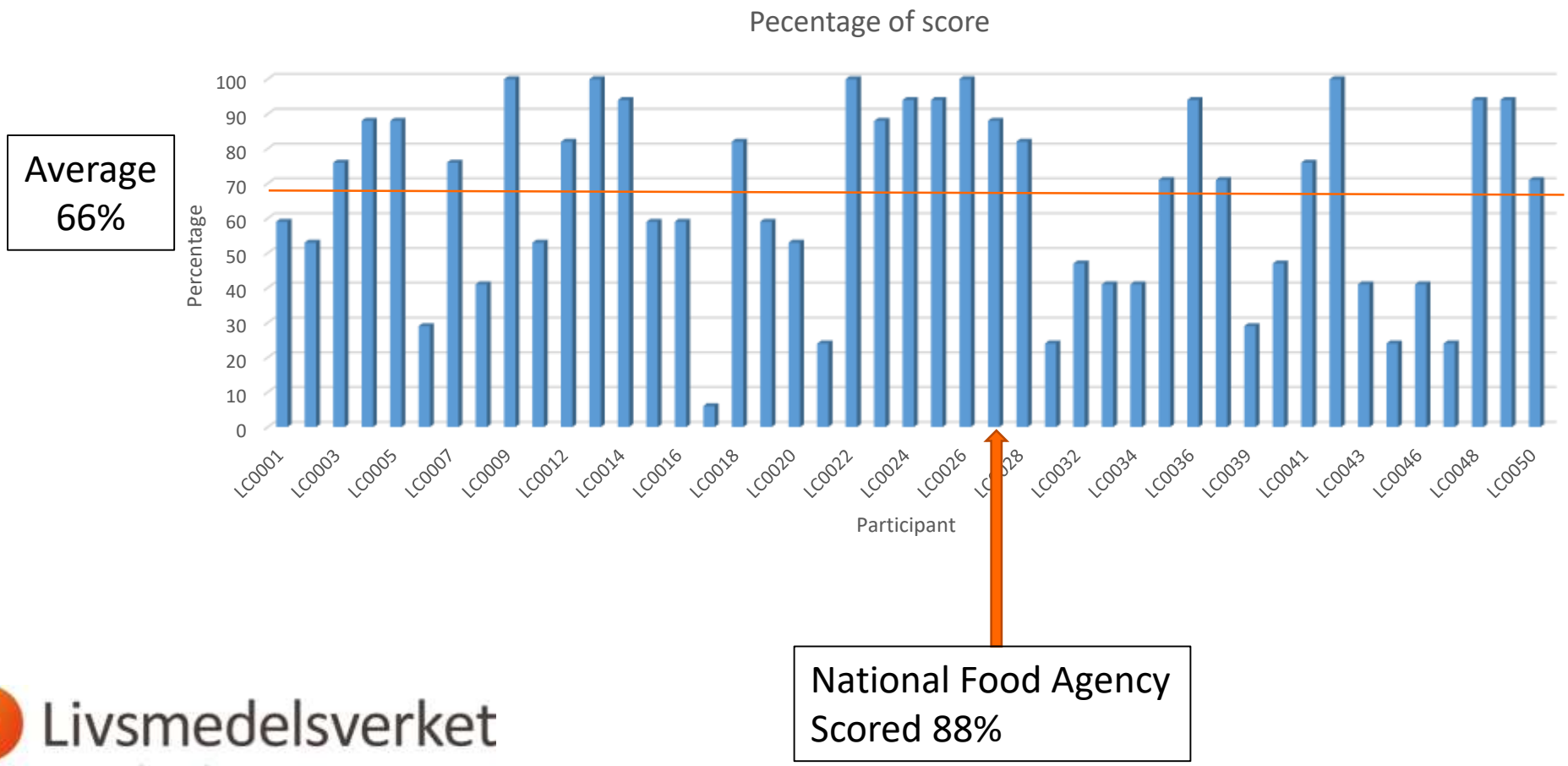
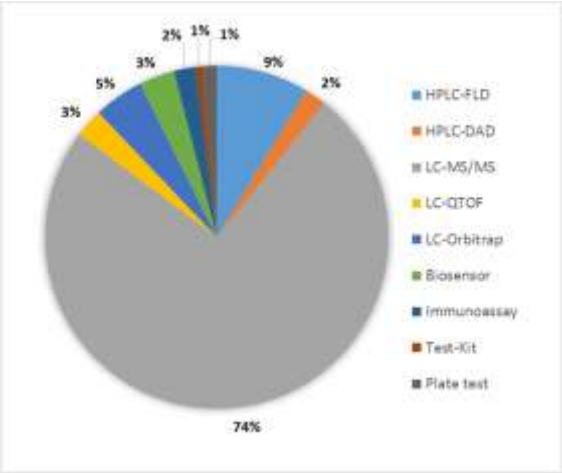
Pharmaceutically Active Drugs in Milk: Proficiency Test MSM1118 (BVL in DE)

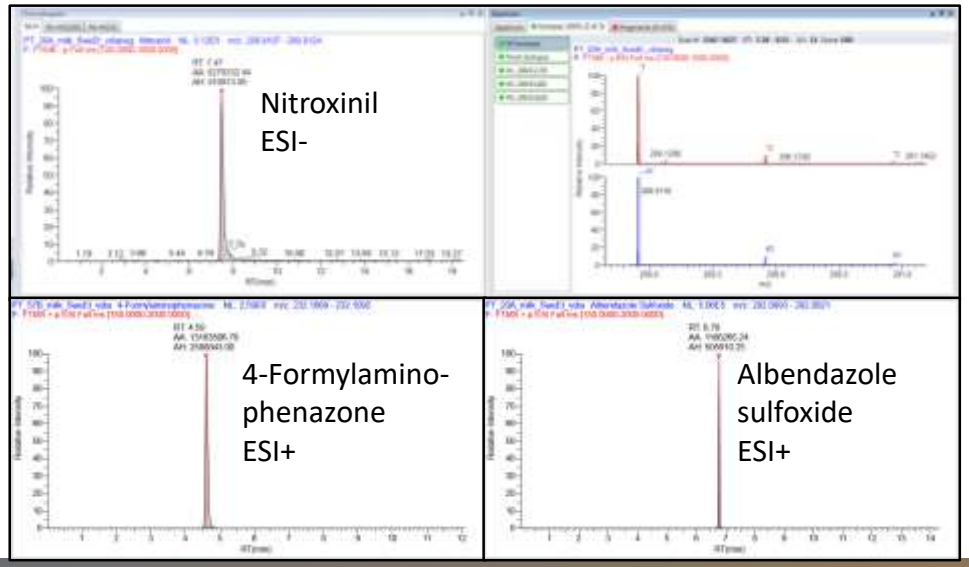


Livsmedelsverket
National Food Agency

Techniques for Screening for drugs in milk

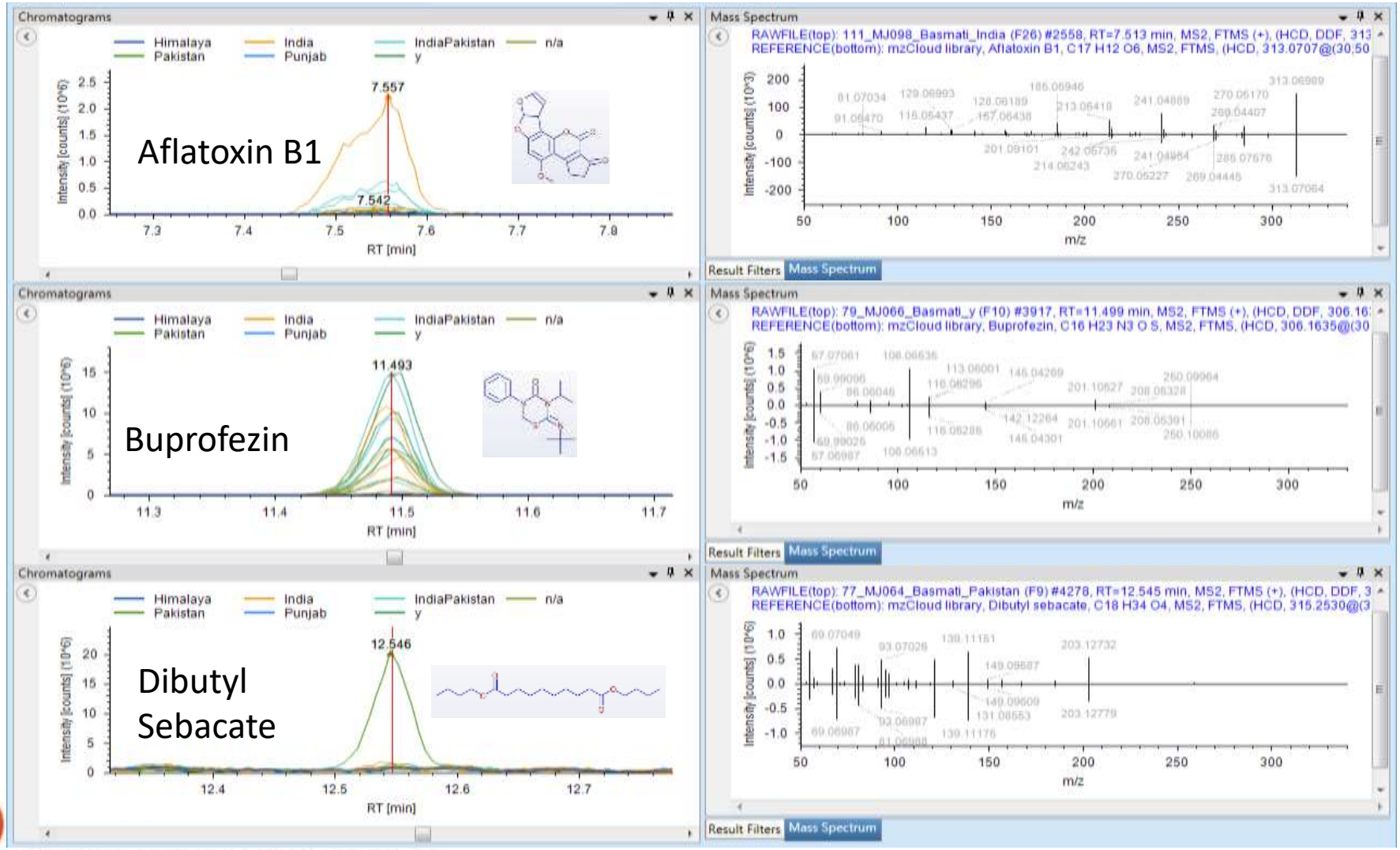


SweEt with Q Exactive Orbitrap



4-aminoantipyrine
4-formylaminophenazone
4-methylaminophenazone
Albendazole sulfone
Albendazole sulfoxide
Amino-albendazole sulfone
Ciprofloxacin
Dimetridazole – false negative
Enrofloxacin
Febendazole
Hydroxy-dimetridazole – false negative
Hydroxy-metronidazole
Levamisole
Metronidazole
Nitroxinil
Oxfendazole
Oxfendazole sulfone
Sulfamethazine/Sulfadimidine
Sulfamethoxazole
Sulfamethoxypyridazine
Triclabendazole
Triclabendazole sulfone
Triclabendazole sulfoxide
Trimethoprim
Tylosin A

Multi class findings in rice



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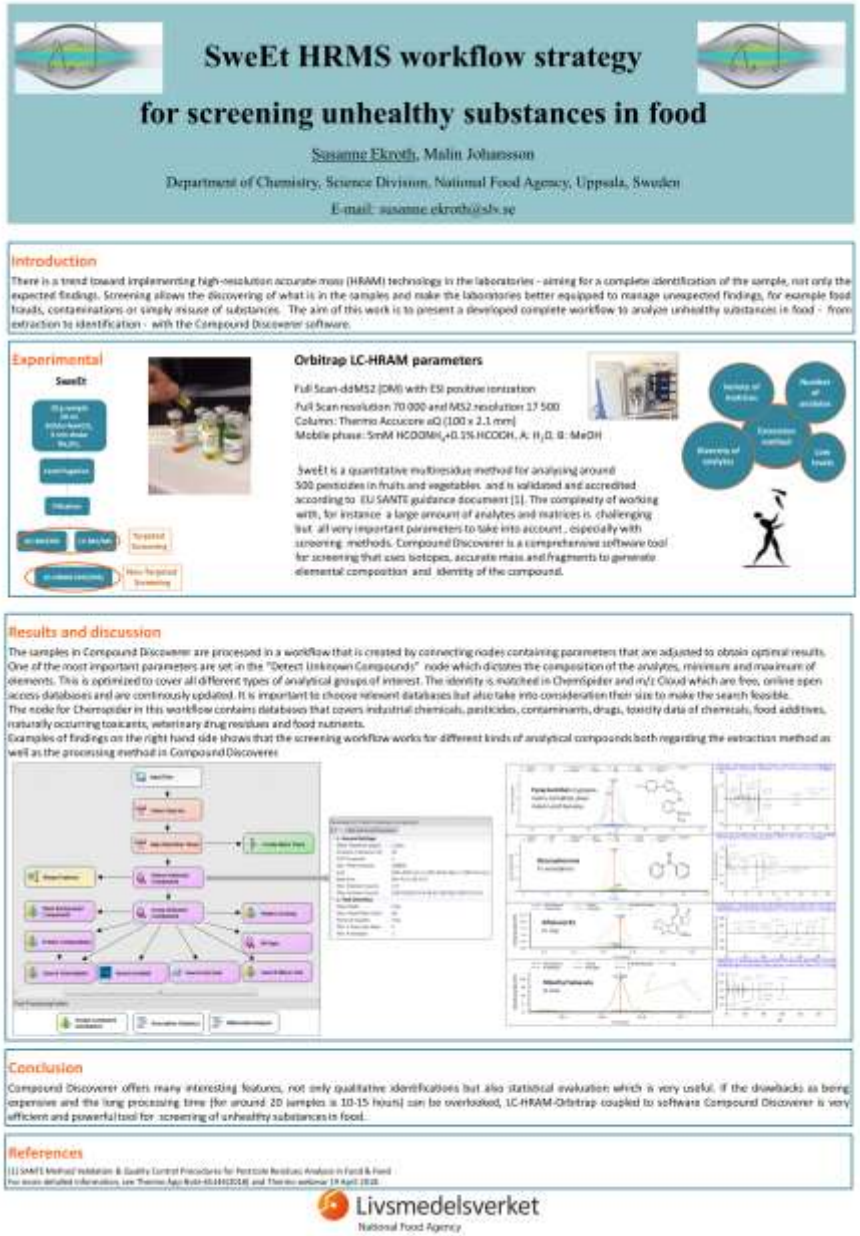
Poster



SweEt HRMS workflow strategy for screening unhealthy substances in food

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Introduction

There is a trend toward implementing high-resolution accurate mass (HRAM) technology in the laboratories - aiming for a complete identification of the sample, not only the expected findings. Screening allows the discovering of what is in the samples and make the laboratories better equipped to manage unexpected findings, for example food frauds, contaminations or simply misuse of substances. The aim of this work is to present a developed complete workflow to analyze unhealthy substances in food - from extraction to identification - with the Compound Discoverer software.

Experimental

SweEt

Orbitrap LC-HRAM parameters

Full Scan-ddMS2 (DIM) with ESI positive ionization

Full Scan resolution 70 000 and MS2 resolution 17 500

Column: Thermo Accucore aQ (100 x 2.1 mm)

Mobile phase: 5mM $HCOONH_4$+0.1% HCOOH, A: H_2O, B: MeOH

SweEt is a quantitative multiresidue method for analysing around 500 pesticides in fruits and vegetables and is validated and accredited according to EU SANTE guidance document [1]. The complexity of working with, for instance a large amount of analytes and matrices is challenging but all very important parameters to take into account, especially with screening methods. Compound Discoverer is a comprehensive software tool for screening that uses isotopes, accurate mass and fragments to generate elemental composition and identity of the compound.

Results and discussion

The samples in Compound Discoverer are processed in a workflow that is created by connecting nodes containing parameters that are adjusted to obtain optimal results. One of the most important parameters are set in the "Detect Unknown Compounds" node which dictates the composition of the analytes, minimum and maximum of elements. This is optimized to cover all different types of analytical groups of interest. The identity is matched in ChemSpider and mz Cloud which are free, online open access databases and are continously updated. It is important to choose relevant databases but also take into consideration their size to make the search feasible.
The node for Chemspider in this workflow contains databases that covers industrial chemicals, pesticides, contaminants, drugs, toxicity data of chemicals, food additives, naturally occurring toxicants, veterinary drug residues and food nutrients.
Examples of findings on the right hand side shows that the screening workflow works for different kinds of analytical compounds both regarding the extraction method as well as the processing method in Compound Discoverer.

Conclusion

Compound Discoverer offers many interesting features, not only qualitative identifications but also statistical evaluation which is very useful. If the drawbacks as being expensive and the long processing time (for around 20 samples is 10-15 hours) can be overlooked, LC-HRAM-Orbitrap coupled to software Compound Discoverer is very efficient and powerful tool for screening of unhealthy substances in food.

References

[1] SANTE Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed
For more detailed information, see Thermo App Note 65144(2014) and Thermo webinar 19 April 2018

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National Food Agency

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Thank you for your attention!

Tuija Pihlström | Martin Isaksson | Ulla Pagard | Eva Flygh | Anna Malmvärn | Anders Edhager