´Organic´ *versus* ´conventionl´ – a novel strategy to identify fraudulent labelling

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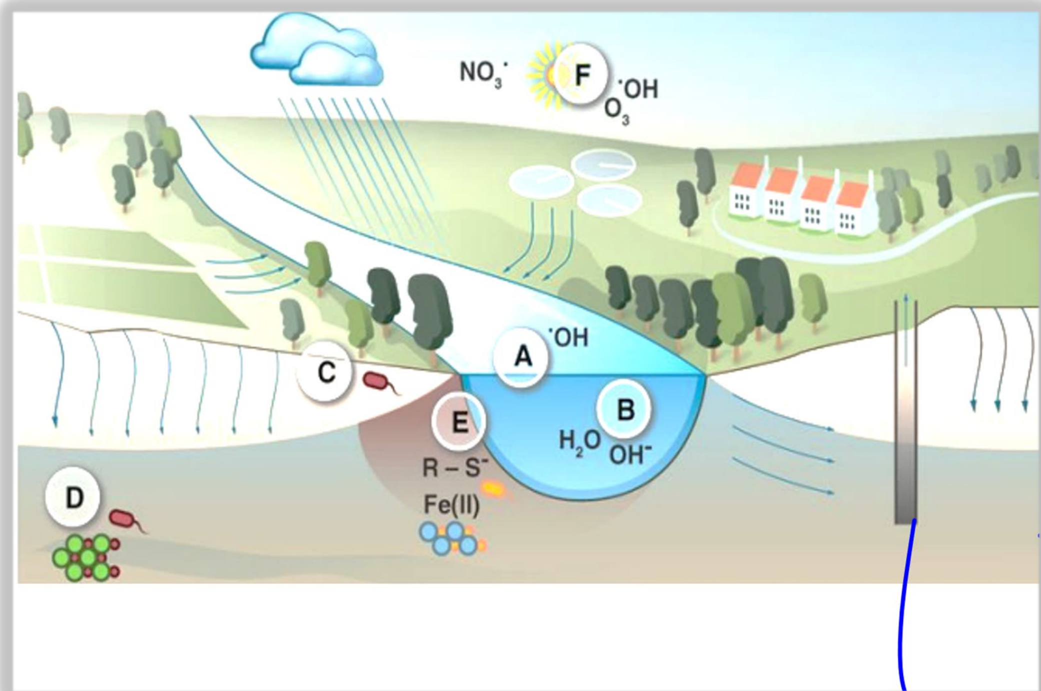
EUCHINASAFE
中欧食品安全

Talk overview

- Pesticide metabolites and transformation products
- Pesticide metabolites as markers of illegal practices on organic crops?
- Occurrence of pesticide metabolites in conventional fruits
- Current analytical approaches in pesticide metabolites analysis
- Food smart phone – screening challenge

What are pesticide metabolites and other transformation products of concern?

- **LEGALY RELEVANT, with a remaining pesticide activity**
- **yet LEGALLY NON-RELEVANT, without well characte-rized pesticide activity and any toxicity**



A Sunlit surface water
e.g., direct phototransformation

B Bulk water body
e.g., hydrolysis

C Vadose zone
e.g., microbial transformation

D Groundwater, oxic
e.g., biotic or abiotic transformation

E Groundwater, anoxic
e.g., reductive transformation

F Troposphere
e.g., indirect phototransformation

Biotransformation of pesticides in plants

Pesticide

different metabolic routes are observed among the representative crops from studies conducted in a similar manner, e.g., foliar spray with similar pre-harvest interval (PHI) and growth stages

Phase I
- **oxidation**
- hydrolysis
- reduction

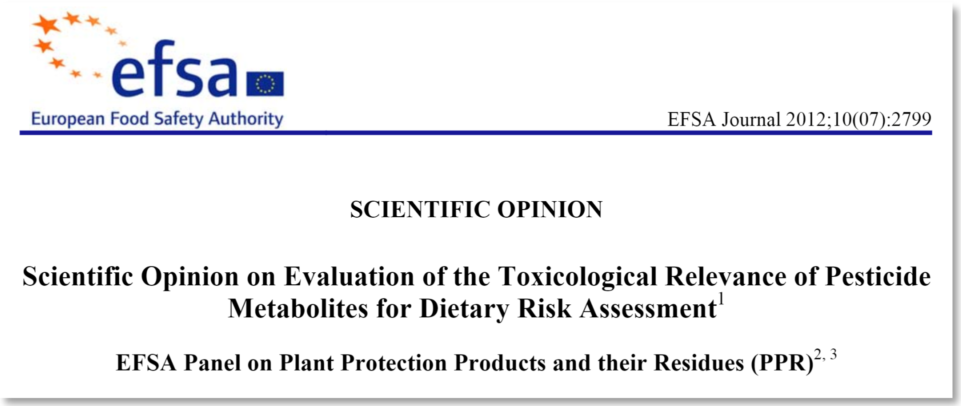
Phase II
- conjugation
  - sugars
  - glutathione
  - aminoacids

Phase III
- conversion of Phase II metabolites into (nontoxic) 2^{o} conjugates
- moving metabolites to vacuole for storage
- Incorporating metabolites into cell wall

Why and how are pesticide metabolites / transformation products studied ?

- **Metabolism experiments**: identification and quantification by methods based on the use of labelled compounds, recently other sophisticated methods
- **Crop field trials:** may also be useful for selecting residue definitions by providing information on the relative and absolute amounts of parent pesticide and metabolites.
- **A pesticide residue is the combination of the pesticide and its metabolites, degradates, and other transformation products.**

Toxicological relevance of metabolites and degradates of pesticide active substances in dietary risk assessment

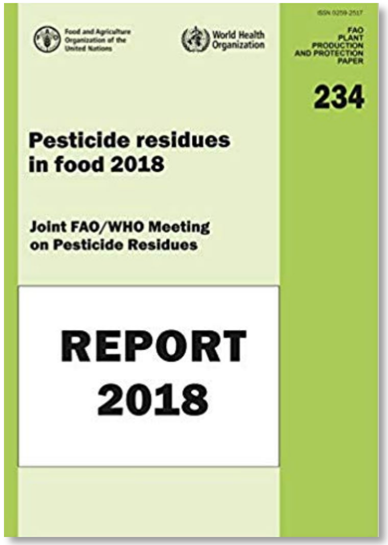

efsa
European Food Safety Authority

EFSA Journal 2012;10(07):2799

SCIENTIFIC OPINION

Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2, 3]



➡ the threshold of toxicological concern (TTC) concept as an appropriate screening tool

➡ critical steps identified in the application of a TTC scheme:

1) the estimate of the level of the metabolite,
2) the evaluation of genotoxicity alerts
3) the detection of neurotoxic metabolites.

Residue definitions (Regulation EC 396/2005)

The residue definition for dietary risk assessment should include compounds of toxicological significance

Residue definitions (Regulation EC 396/2005)

Residue...	defined as the sum of:
amitraz	amitraz including the metabolites containing the 2,4 -dimethylaniline moiety expressed as amitraz
aldicarb	aldicarb, its sulfoxide and its sulfone, expressed as aldicarb
carbendazim and benomyl	benomyl and carbendazim expressed as carbendazim
carbofuran	carbofuran (including any carbofuran generated from carbosulfan, benfuracarb or furathiocarb) and 3-OH carbofuran expressed as carbofuran
daminozide	daminozide and 1,1-dimethyl-hydrazine (UDHM), expressed as daminozide
dazomet	methylisothiocyanate resulting from the use of dazomet and metam
disulfoton	disulfoton, disulfoton sulfoxide and disulfoton sulfone expressed as disulfoton
ethofumasate	ethofumesate, 2-keto–ethofumesate, open-ring-2-keto-ethofumesate and its conjugate, expressed as ethofumesate
fenamiphos	fenamiphos and its sulphoxide and sulphone expressed as fenamiphos
fluroxypyr	fluroxypyr, its salts, its esters, and its conjugates, expressed as fluroxypyr
folpet	folpet and phtalimide, expressed as folpet
fosethyl-Al	fosetyl, phosphonic acid and their salts, expressed as fosetyl
haloxyfop	haloxyfop, haloxyfop-ethoxyethyl and haloxyfop-methyl expressed as haloxyfop
oxydemeton-methyl	oxydemeton-methyl and demeton-S-methylsulfone expressed as oxydemeton-methyl
quintozene	quintozene and pentachloro-aniline expressed as quintozene
tolylfluanid	tolylfluanid and dimethylaminosulfotoluidide (DMST) expressed as tolylfluanid

What about those ´legally non-relevant´ metabolites?

AUTHENTICATION

of organic crops (?)

Award: article of the month

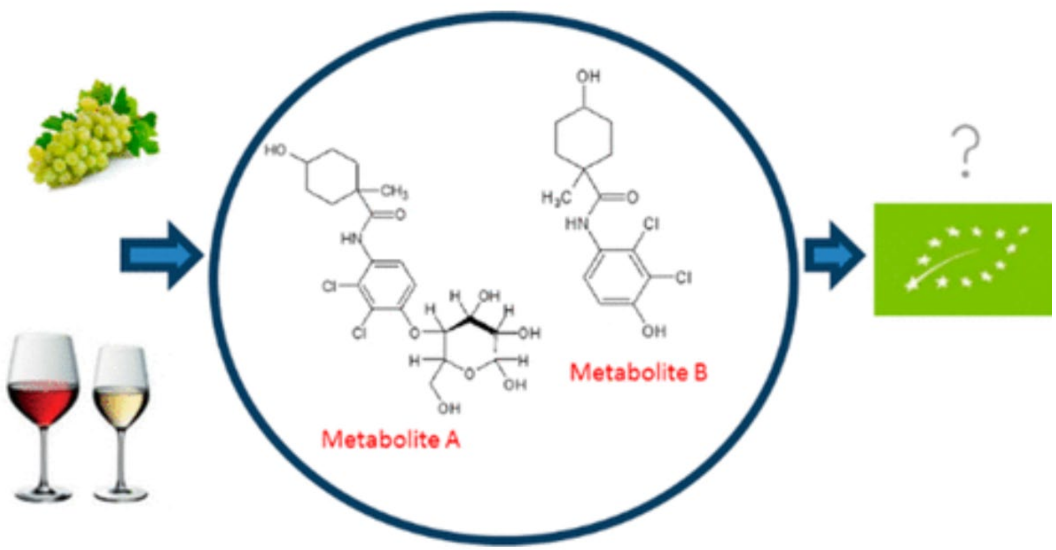
Journal of Agricultural and Food Chemistry

2019, 67, 22m
6102 - 6115

American Chemical Society

Can Occurrence of Pesticide Metabolites Detected in Crops Provide the Evidence on Illegal Practices in Organic Farming?

Schusterova D, Suchanova M, Pulkrabova J, Koourek V, Hajslova J



How is ´organic food´ defined ?

→ **´food originated from organic production´**

Note: that only production process and conditions are defined – not the product features (parameters)

REGULATION (EC) No 834/2007 on organic production and labelling of organic products:

*„**Organic production** is a <u>system of farm management and food production</u> that combines best environmental practices,… and a production method in line with the preference of certain consumers for products produced <u>using natural substances and processes</u>.“*

Legislation on organic production

REGULATION (EC) No 889/2008 laying down rules for the implementation of Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.

Rules on production, preservation, processing, packaging, transport and storage of organic products

Restrictions on:

- fertilizers, soil conditioners and nutrients,
- **PESTICIDES***,
- feed materials, feed additives used in animal nutrition
- products for cleaning and disinfection,
- products and substances for use in production of processed organic food (food additives),
- processing aids

ANNEX II: ´Pesticides´ permitted in organic farming

„Where plants cannot be adequately protected from pests and diseases by measures provided for in Article 12 of Regulation 834/2007/EC, <u>only products referred to in Annex II may be used in organic production</u>. Operators shall keep documentary evidence of the need to use the product.“

ANNEX II: ´Pesticides´ permitted in organic farming

Name	Description, compositional requirement, conditions for use
Azadirachtin extracted from *Azadirachta indica* (Neem tree)	
Plant oils	All uses authorised, except herbicide.
Pyrethrins extracted from *Chrysanthemum cinerariaefolium*	
Pyrethroids (only deltamethrin or lambdacyhalothrin)	Only in traps with specific attractants; only against *Bactrocera oleae* and *Ceratitis capitata* Wied.
Quassia extracted from *Quassia amara*	Only as insecticide, repellent.
Repellents by smell of animal or plant origin/sheep fat	Only on non-edible parts of the crop and where crop material is not ingested by sheep or goats.
Micro-organisms	Not from GMO origin.
Spinosad	

Let´s introduce the background of the authentication study in a more detail

Challenge: bio-wines authentication

EPRW 2016, biowinesLim assool 23.-26.9.2016

Bacgreound of study:
Growing popularity of ´bio-wines´ has raise a demand for comntrol of compliance of organic farming practices usewd in grapes production (Commission Regulation 889/2008).

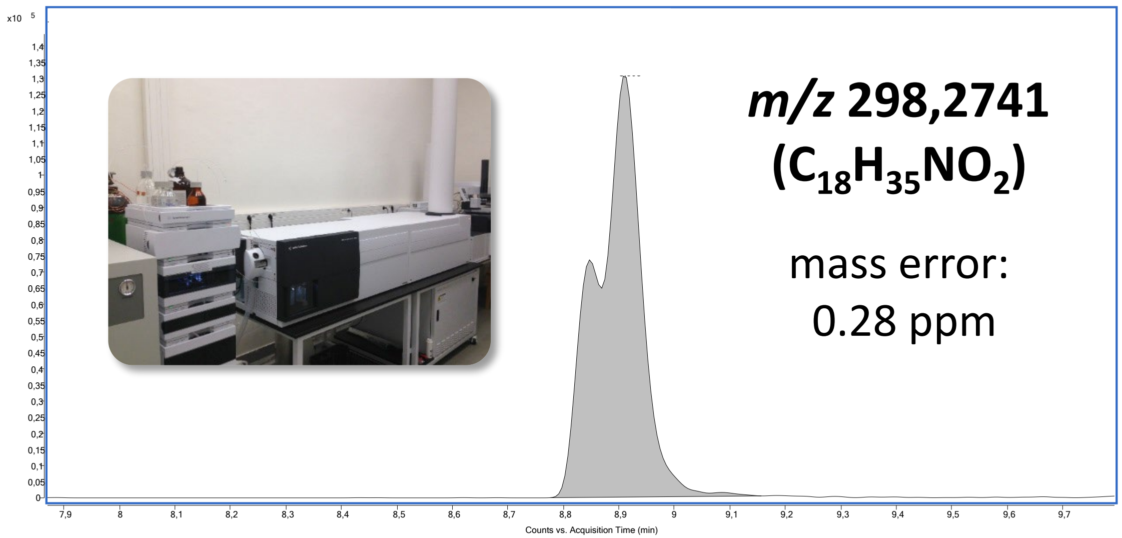
PROBLEM: occurrence of unauthorized pesticide residues close to 0.01 mg/kg in ´organic´ grapes found by Control Authority, however, how to interpret it?

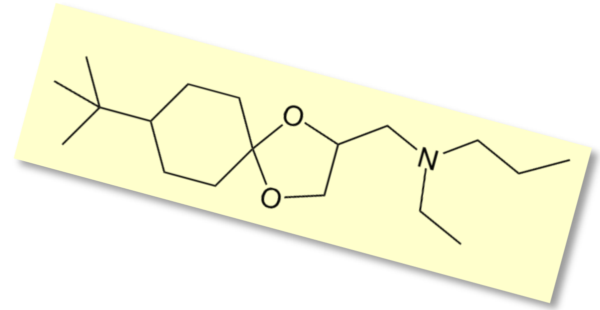
- **Accidental contamination e.g. through atmospheric transport**

or

- **Illegal use of pesticide preparations**

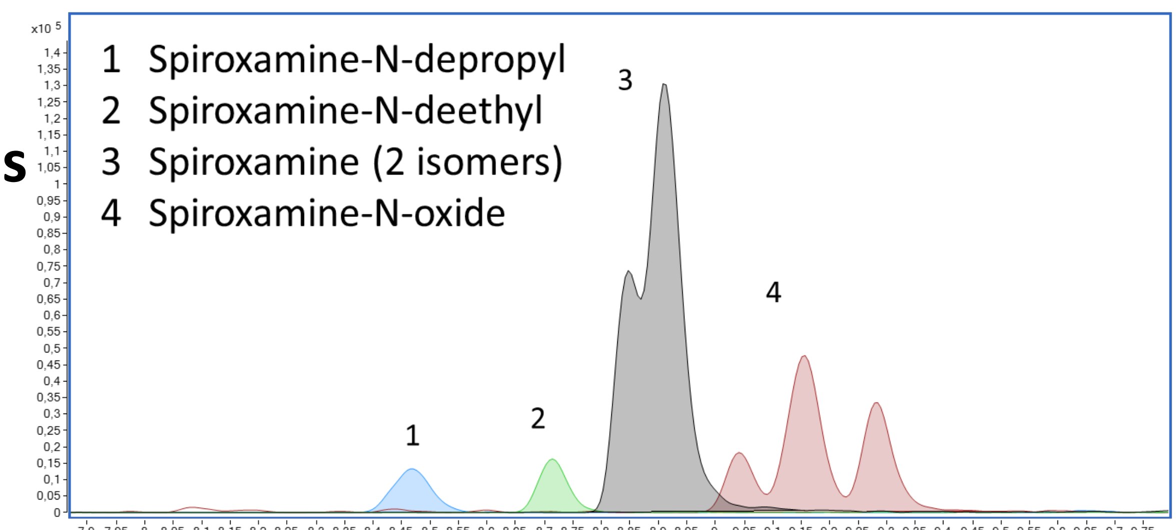
Example of difficult decision: is the wine organic?





Spiroxamine – 0.01 mg/kg
→ „BIO“ MLR

1st step ✓



Spiroxamine + metabolites
→ this does not comply with „BIO“ MLR

2nd step X

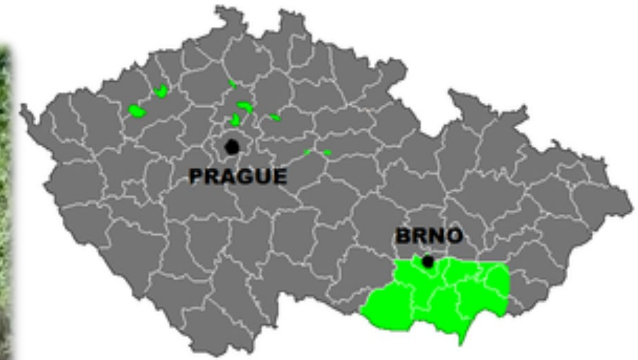
Case study:

Monitoring of pesticide residues and their metabolites across a wine production chain

Field experiments (2016 – 2018)

Leaves and grapes sampled by Central Institiue for Agriculture Testing, Brno

ÚKZÚZ



↑

Moravia vineyards

A. Organic vineyard – certified

B. Conventional vineyard

Fungicides used for treatment →

Dimethomorph
Fenhexamid
Iprovalicarb
Pyraclostrobin
Quinoxyfen
Spiroxamine
Tebuconazole
Triadimenol
Trifloxystrobin

Folpet
Fosetyl-Al
Iprodion
Mancozeb
Metiram

Persistence of fungicides used for protection of *Vinis vinifera* plants (grapes)

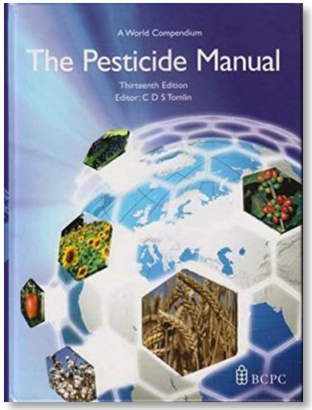
Pesticide	LOQ mg/kg	Rheinischer Riesling		
		$C_{starting}$ (mg/kg)	C_{final} (mg/kg)	% decrease
Fenhexamid	0,002	0,802	0,192	76
Spiroxamine	0,001	0,048	0,024	50
Difenoconazole	0,001	0,017	0,009	47
Pyrimethanil	0,001	0,189	0,107	43
Iprovalicarb	0,001	0,015	0,011	27
Fluopicolide	0,001	0,210	0,172	18
Pyraclostrobin	0,001	0,006	0,005	17
Dimethomorph	0,001	0,088	0,083	6
Meptyldinocap	0,005	<0,005	<0,005	–

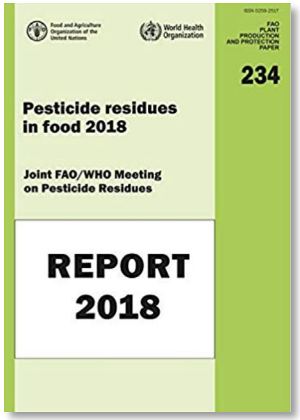
Pesticide	LOQ mg/kg	Cabernet Sauvignon		
		$C_{starting}$ (mg/kg)	C_{final} (mg/kg)	9 weeks % decrease
Fenhexamid	0,002	0,227	0,017	93
Spiroxamine	0,001	0,981	0,091	91
Difenoconazole	0,001	0,028	0,007	75
Pyrimethanil	0,001	0,249	0,078	69
Iprovalicarb	0,001	0,341	0,126	63
Fluopicolide	0,001	0,045	0,017	62
Pyraclostrobin	0,001	0,043	0,018	58
Dimethomorph	0,001	0,009	0,005	44
Meptyldinocap	0,005	<0,005	<0,005	–

Sources employed for Database of pesticide metabolites construction

JMPR documents, EFSA opinions, Pesticide manual, scientific literature…

European Food Safety Authority



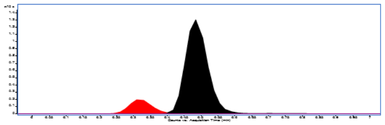


HRMS/MS screening and confirmation

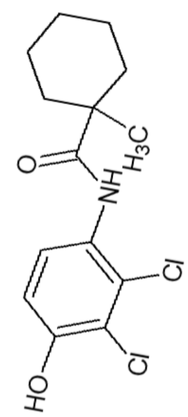
Accurate mass (± 5 ppm), isotope profile

Interpretation of MS/MS fragments

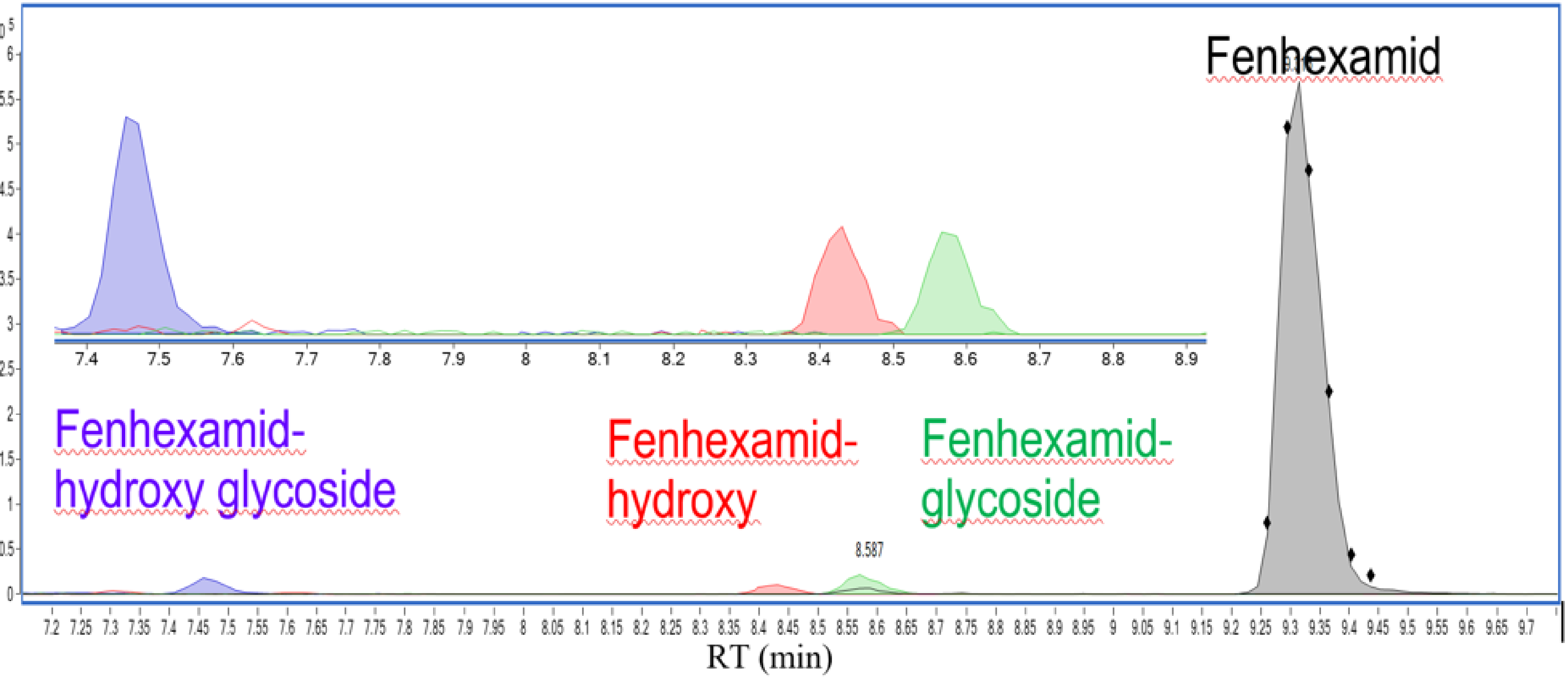
- Diagnostic fragments
- Fragments complying to parent molecule
- Fragments characterizing metabolic transformation
- Neutral losses



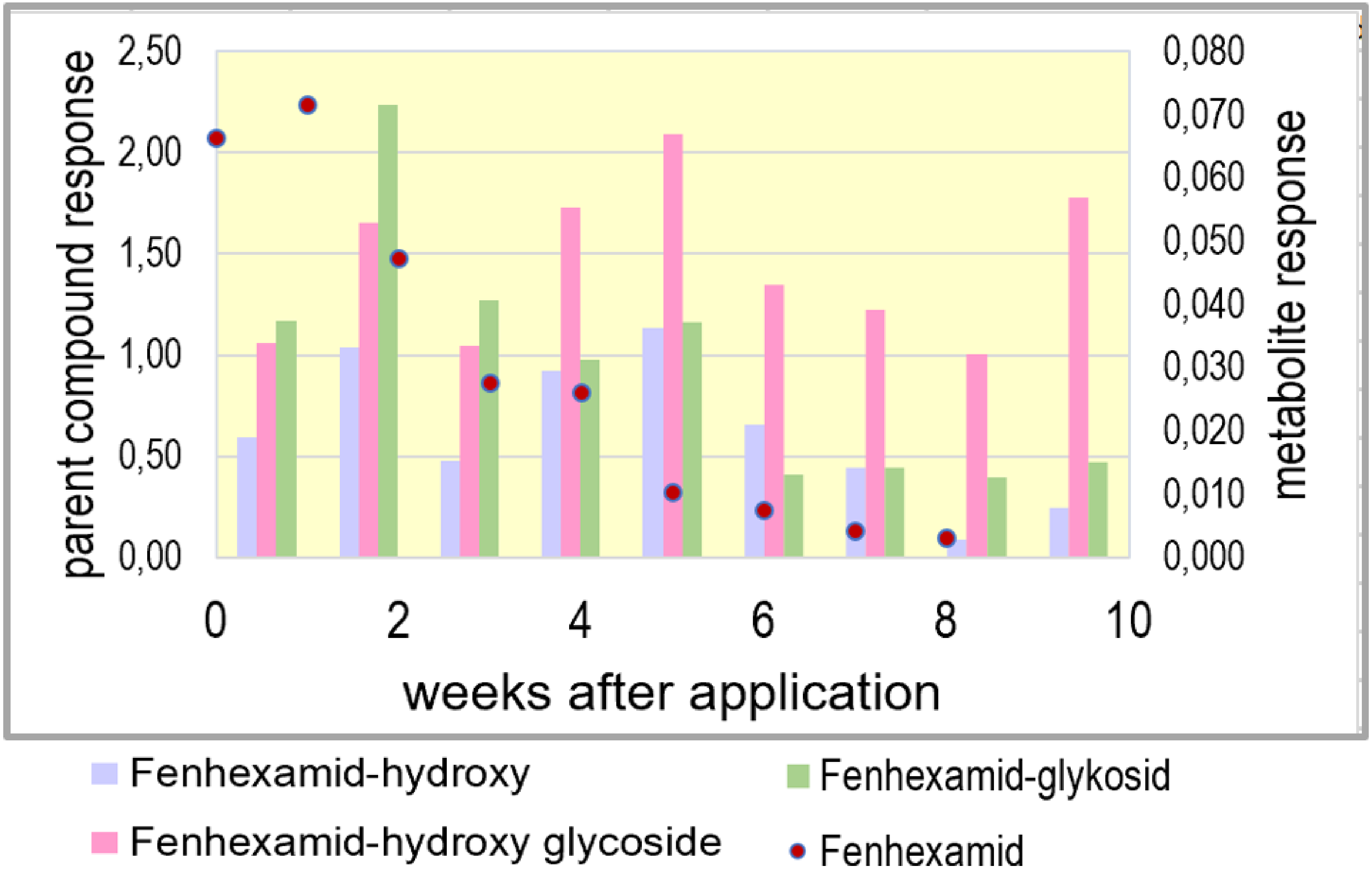
Search for fenhexamid and its metabolites – an example of one of database items

Pesticide - parent compound	Structure, elemental formula, accurate mass (Da)	Metabolites	Elemental formula	Accurate mass (Da)
Fenhexamid	 $C_{14}H_{17}Cl_2NO_2$ 301,0636	Fenhexamid-glykoside	$C_{20}H_{27}Cl_2NO_7$	463,1165
		Fenhexamid-hydroxy	$C_{14}H_{17}Cl_2NO_3$	317,0585
		Fenhexamid-hydroxy glykoside	$C_{20}H_{27}Cl_2NO_8$	479,1114
		Difenhexamid	$C_{28}H_{32}Cl_4N_2O_4$	600,1116

Search for fenhexamid and its metabolites – an example of one of database items



Search for fenhexamid and its metabolites – an example of one of database items



Number of metabolites detected across wine production chain

PESTICIDE	METABOITE	MATRIX					
		leaves		grapes		wine	
		RR	CS	RR	CS	RR	CS
Difenoconazole	*Difenoconazole CGA 205374*	✓	✓	✓	✓	×	×
Dimethomorph	*Dimethomorph hydroxy*	✓	✓	×	×	×	×
	Dimethomorph-demethyl	✓	✓	✓	✓	✓	✓
	Dimethomorph-demethyl glycoside	×	×	✓	✓	×	×
Fenhexamid	*Fenhexamid-hydroxy*	✓	✓	✓	✓	✓	✓
	Fenhexamid-hydroxy glycoside	✓	✓	✓	✓	✓	×
	Fenhexamid-glykosid	×	×	×	✓	×	×
Fluopicolide	*Fluopicolide hydroxy*	✓	✓	×	✓	×	×
Iprovalicarb	*Iprovalicarb-hydroxy*	✓	✓	✓	✓	×	✓
	Iprovalicarb-hydroxy glycoside	✓	✓	✓	✓	✓	✓

PESTICIDE	METABOITE	MATRIX					
		leaves		grapes		wine	
		RR	CS	RR	CS	RR	CS
Pyraclostrobin	*Pyraclostrobin-desmethoxy*	✓	✓	✓	✓	×	×
	Pyraclostrobin-hydroxy	✓	✓	×	✓	×	×
	Pyraclostrobin-hydroxy glycoside	✓	×	×	×	×	×
Pyrimethanil	*Pyrimethanil hydroxy*	✓	✓	×	✓	✓	✓
	Pyrimethanil-hydroxy glycoside	✓	✓	×	✓	×	×
	Pyrimethanil-metabolit A	×	✓	✓	✓	✓	✓
	Pyrimethanil-metabolit B	×	✓	✓	✓	✓	✓
Spiroxamine	*Spiroxamine-N-desethyl*	✓	✓	✓	✓	✓	✓
	Spiroxamine-N-despropyl	✓	✓	✓	✓	✓	✓
	Spiroxamine-N-oxid	✓	✓	✓	✓	×	×

And what about the bio-wines at the current market?

Monitoring of bio-wines at EU market

Samples analyzed for the presence of pesticides and their metabolites:

- 28 white bio-wines
- 29 red bio-wines

No pesticide residues / their metabolites detected in 72 % bio wines

- **19% of samples contained detcetable pesticide residues together with some of their metabolites**
- **7% of samples contained more tha one pesticide (parent compound)**
- **4% of samples contained only detectable pesticide metabolites**
- **1% of samples contaned pesticide residues not registered in EU**

The most frequently detected pesticides: fluopyramu, myclobutanilu a tebufenozidu and relevant metabolites:

fluopyram-hydroxy
myclobutanil-hydroxy
tebufenozide-hydrox

Monitoring study on the occurrence of pesticide metabolites in various (conventional) crops might be interesting…

Pesticide metabolites in fruit - a survey
(35 samples per each commodity)

- **Grapes and wine:**
  - **37 pesticide residues detected, 98 metabolites searched → 32 metabolites detected and identified (relevant to 18 pesticides)**

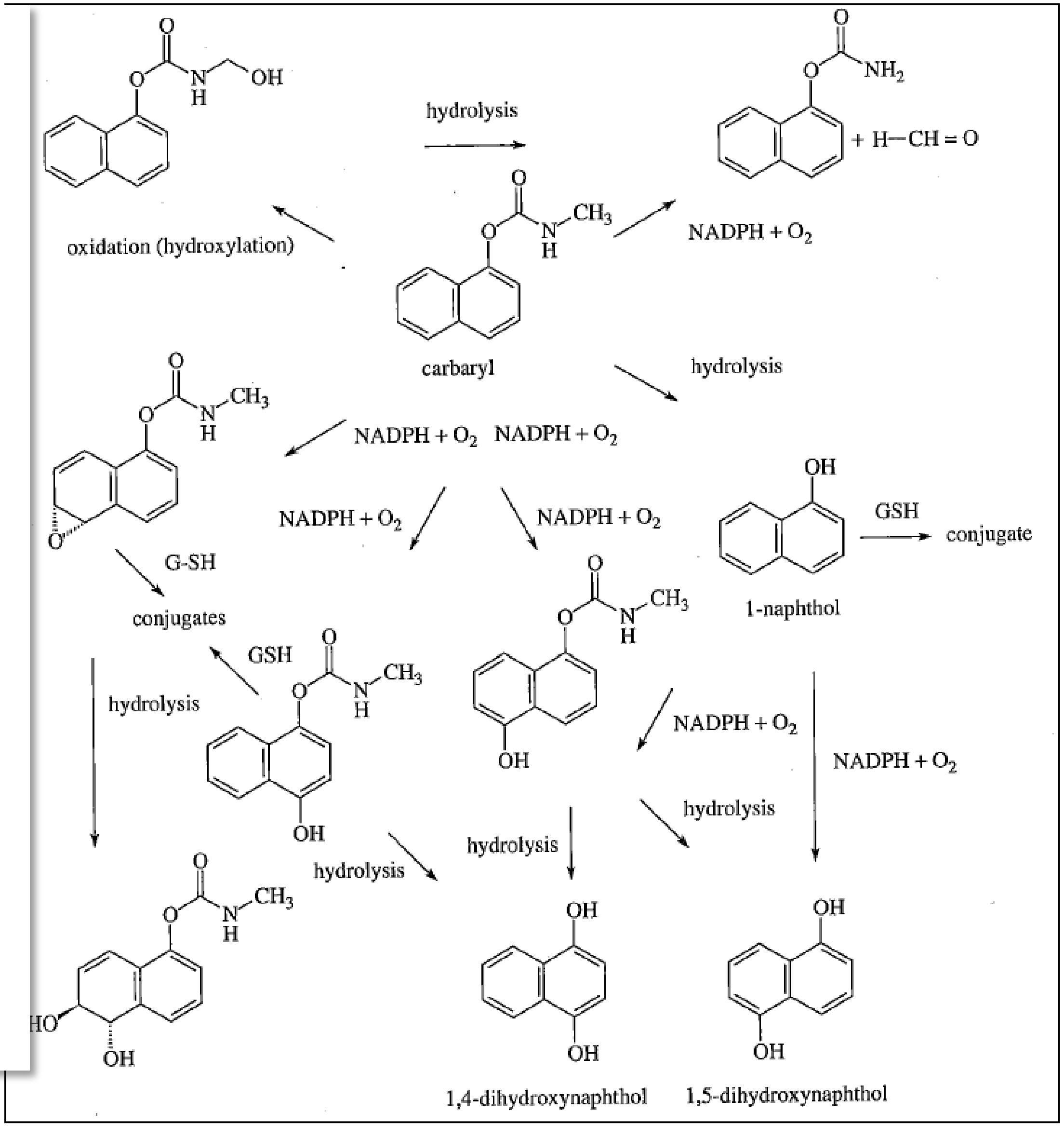
- **Apples:**
  - **45 pesticide residues detected, 114 metabolites searched → 29 metabolites detected and identified (relevant to 26 pesticides)**

- **Citrus fruit:**
  - **13 pesticide residiues detected, 30 metabolites searched → 8 metabolites detected and identified (relevant to 5 pesticides)**

PESTICIDE METABOLITES: analytical strategies

Challenges in pesticide metabolites analysis

- Number of metabolites originates from parent pesticide → very low conc.
- Most of them are fairly more polar compared to parent compound
- The dynamics of individual metabolites origination is not known
- ***Analytical standards are not available***



Current state-of-the-art in pesticide metabolites LC-MS analysis

Food Analytical Methods (2018) 11:1591–1607
https://doi.org/10.1007/s12161-017-1143-4

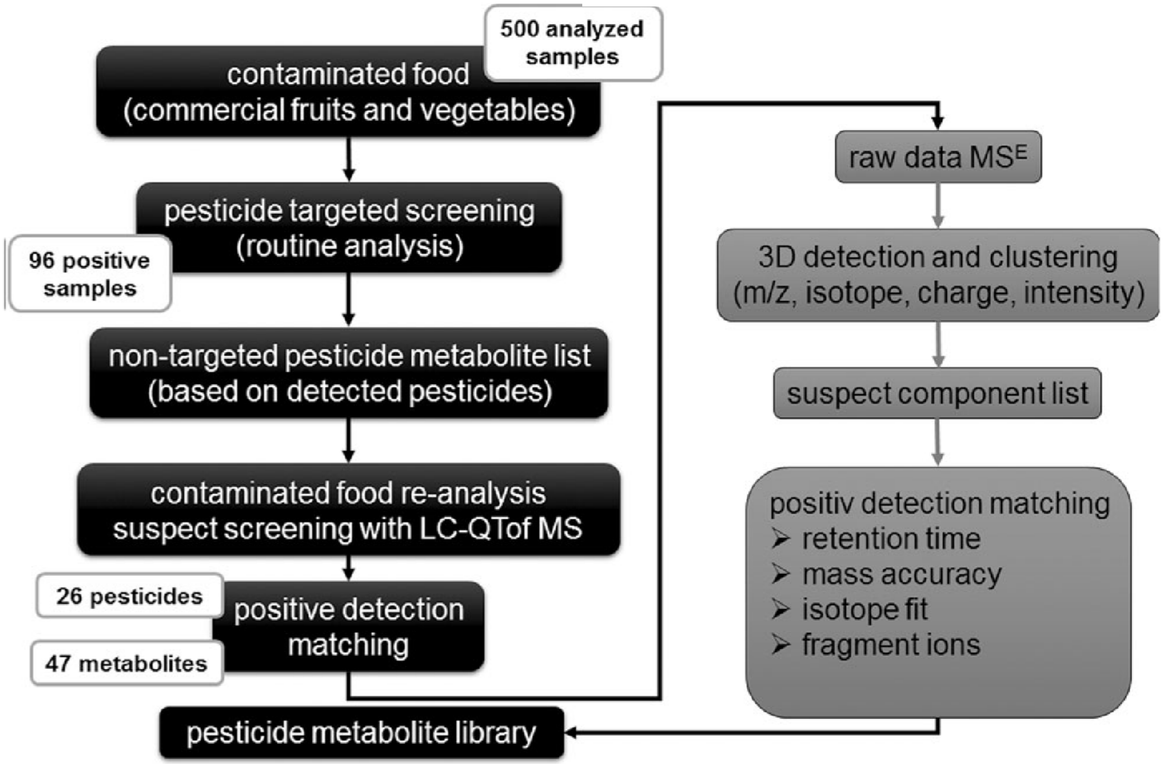
Metabolites and parent compounds analysed in a separate runs (LC-MSPMS and **LC-HRMS)**

METABOLITES
(648 compounds):

Extraction performed by MeOH – water (50:50, v/v)

Clean-up by SPE and passing the sample through the syringe filter

Time consuming and rather labour intensive procedure



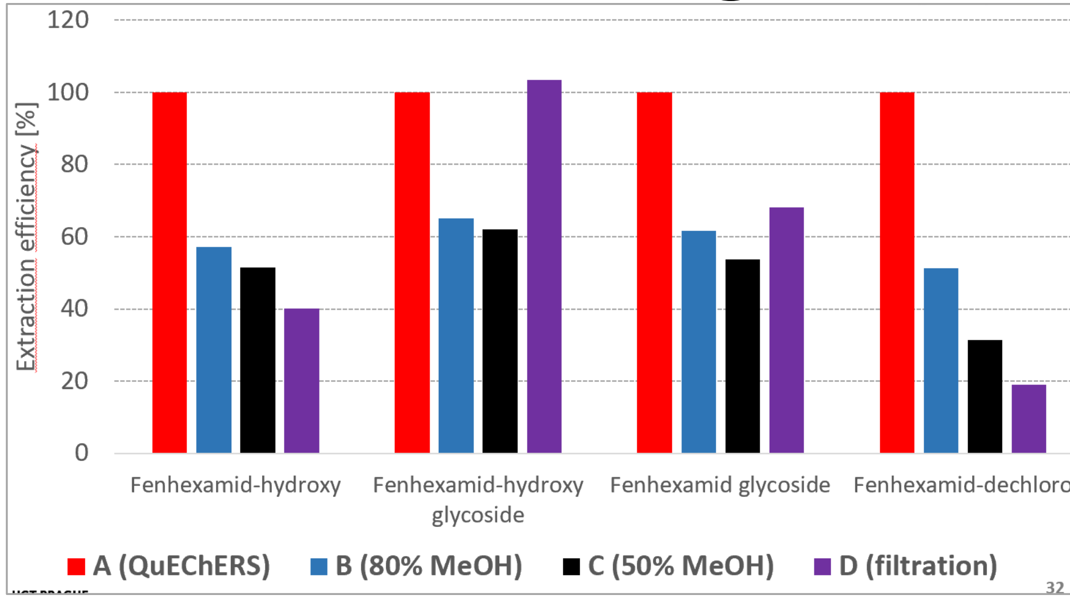
Development of a Suspect Screening Strategy for Pesticide Metabolites in Fruit and Vegetables by UPLC-Q-Tof-MS

Anna Bauer[1] · Jens Luetjohann[1] · Sascha Rohn[2] · Eckard Jantzen[1] · Jürgen Kuballa[1]

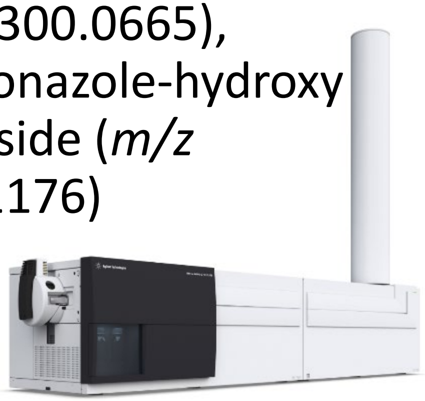
Is it possible to involve multiple pesticide metabolites into current multiresidue method ?

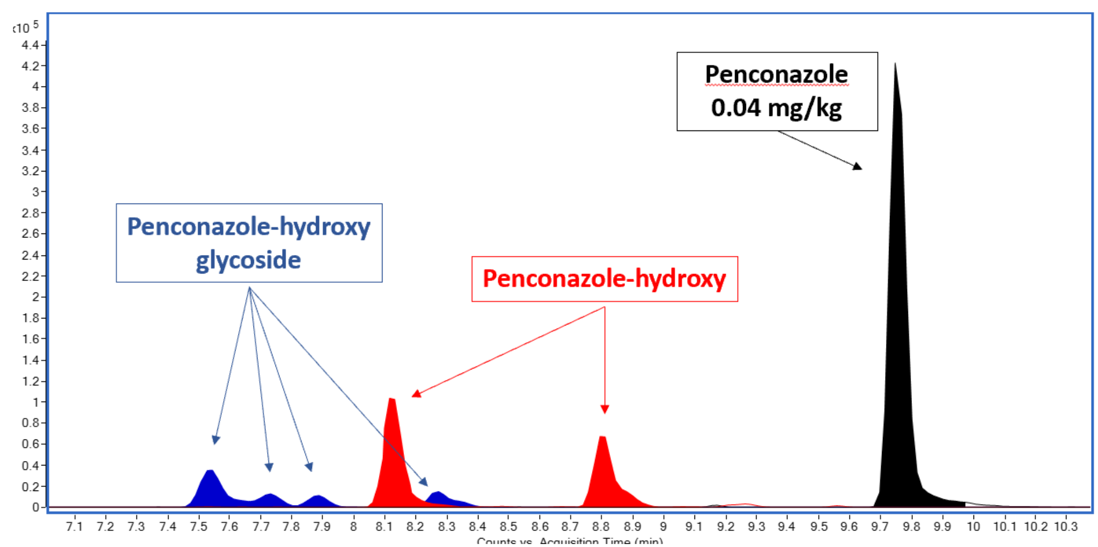
Multi-analyte approach, parent pesticides and their metabolites in a single run

QuEChERS extraction provided most intensive signals (less matrix effects?)



- **Penconazole in grapes** (*m/z* 284.0721), penconazole-hydroxy (*m/z* 300.0665), penconazole-hydroxy glycoside (*m/z* 462.1176)





CONCLUSIONS

- Detection of fraud (illegal treatment of crop by pesticides) is possible based on pesticide metabolites screening, standards are needed for quantification

  However, intensive research of pesticide metabolism is needed to identify reliable markers for particular crop

- Most of metabolites (pesticide treatment markers) might be incorporated into current multi-residue methods

- Possible interference of dietary pesticide metabolites with biomonitoring studies should be assessed (risk of exposure overestimation)