529-0043-00L
Analytical Strategy
ETH Zurich

Analysis of pesticides in food

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Agenda

- Central laboratory of Coop
- Department of trace analysis @ Coop
- What is important in the job?
- Question 1: Analyze: what? when? targets?
- Question 2: Quantiation: Ideal vs reality
- Question 3: Confirmation: How and when? + examples
- Question 4: Retro-analysis: Potential problems?
- Question 5: Glyphosat: Analytical approach?
- Question 6: Assessment of residues

Coop Central Laboratory

- Responsible for the analytical work and related questions for the whole Coop group.
- Together with quality assurance (total ca. 60 persons)
  ➔ whole QM-team in the same building
- Located in Pratteln (near Basel)
- Laboratory: 35 employees, 5 departments
- ISO 17025 accreditation
- No contract lab
- Broad range of analytical methods and samples (food, feed, non-food):
  ➔ 815 active methods covering 2467 parameters in e.g.
  - microbiology
  - GMO
  - food composition
  - physical & visual properties
  - food additives, vitamines
  - mycotoxins
  - **trace analysis**
  - non-food testing

Coop Central Laboratory

Department of trace analysis

- Staff: 8 (6 technicians, 2 academics)
- Instrumentation:
  LC-Q-ToF, 2 LC-MS/MS, GC-MS/MS, 2 GC-MS
  GC-FID, GC-PFPD, GC-sniff
  ICP-OES, GFAAS
- Main analyses: - veterinary drugs
  - pesticides
  - heavy metals
  - plasticizers
  - polycyclic aromatic hydrocarbons
  - illegal dyes
  - taints / off-flavours
- Main samples come from our competence labels, e.g.
  - organic fruits and vegetables
  - Naturafarm & Bio meat and fish
  - Naturaline textile

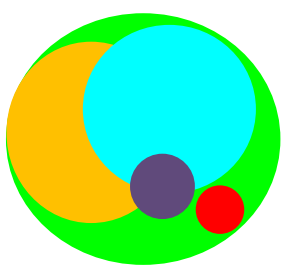
Range of samples

What is important in the job?

- Legal aspects
- Multidisciplinary approaches
- Explaining and presenting
- People: understand them, motivate them, lead them
- Efficiency: a lot is about time and money!
- Network
- Stay curious

- And of course: knowledge!

Question 1
Analyse: What? When? How?



Question 1
Analyse: What? When? How?

- Residue data, check data bases:
  - ➔ how often is the compound found?
  - ➔ on what crops?
  - ➔ country of origin? Important for supply chain?
  - ➔ MRL exceedencies?
  - ➔ Are there registered applications for the compound?
- Authorisation of use? On what crops?
- RASFF alerts? https://webgate.ec.europa.eu/rasff-window/portal/
- Season vs climate vs susceptibility of crop
- Amenable to multi-methods? ↔ efficiency?!
- Long-term experience / own data
- Information from your network
- Public interest / pressure
- Relevance to company / economic factors
- Supplier: performance in preceeding years; new?
- …
- … … but also do the unexpected!

Question 1: Examples

- Lettuce in January / February
- Strawberries from cold and wet summers
- Grapes from harvests with havy rainfall
- Vegetables from Thailand

Lettuce, France, February 2011

CS46420 PestizideQuEChLC

Pestizide QuEChERS LC	nw	
Acetamiprid	0.053	mg/kg
Iprodion	0.191	mg/kg
Propamocarb	3.46	mg/kg

CS46430 PestizideQuEChGC

Pestizide QuEChERS GC	nw	
Bifenthrin	0.107	mg/kg
Cyprodinil	0.343	mg/kg
Fludioxonil	0.461	mg/kg
lambda-Cyhalothrin	0.0459	mg/kg
Pencycuron	0.0112	mg/kg
Pyrimethanil	0.456	mg/kg

CS46460*

Folpet	0.139	mg/kg

Hot-Chili, Thailand, March 2010

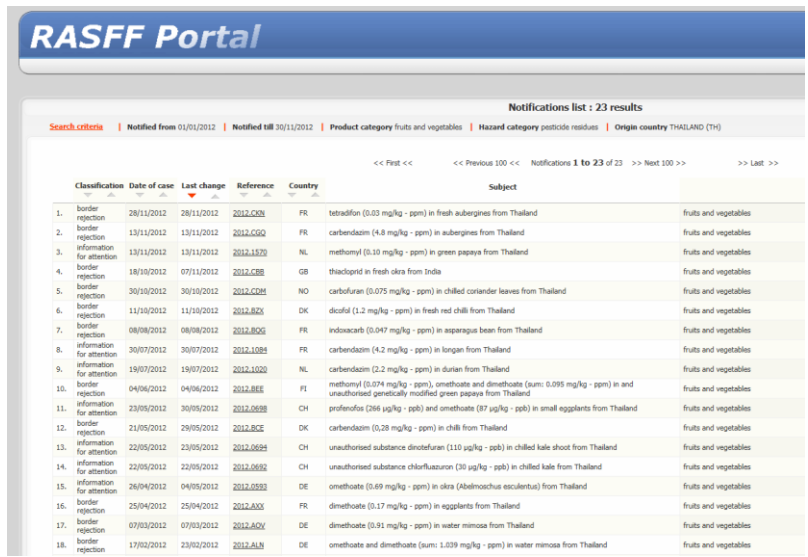
Methode / Messgrösse	Resultat	Einheit	Coop-Norm	Toleranzwert	Grenzwert oder Textnorm
Pestizide/Begasungsmittel					
CS46420 PestizideQuEChLC					
Pestizide QuEChERS LC	nw				
Carbendazim	0.205	mg/kg		max. 0.100	
Methomyl	0.0352	mg/kg		max. 0.2000	
Profenofos	1.21	mg/kg			max. 0.05 (EU Höchstwert)
Summe Methomyl	0.0352	mg/kg		max. 0.2000 (inkl. Thiodicarb)	

Ergebniskommentar zu Pestizide QuEChERS LC:
Das Untersuchungsspektrum umfasst 85 Wirkstoffe
Bestimmungsgrenze: 0.01 mg/kg
Ergebniskommentar zu Summe Methomyl: Summe von Methomyl und Thiodicarb berechnet als Methomyl

CS46430 PestizideQuEChGC

Pestizide QuEChERS GC	nw				
Chlorpyrifos(-ethyl)	0.171	mg/kg		max. 0.050	
Cypermethrin	0.0288	mg/kg		max. 0.5000	

Question 1: RASFF



RASFF Portal

Notifications list : 23 results

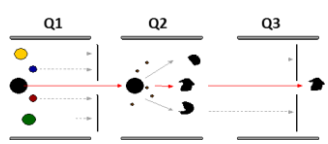
Search criteria | Notified from 01/01/2012 | Notified till 30/11/2012 | Product category fruits and vegetables | Hazard category pesticide residues | Origin country THAILAND (TH)

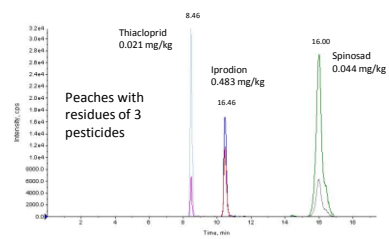
<< First << << Previous 100 << Notifications 1 to 23 of 23 >> Next 100 >> >> Last >>

	Classification	Date of case	Last change	Reference	Country	Subject	
1.	border rejection	28/11/2012	28/11/2012	2012.CKN	FR	tetradifon (0.03 mg/kg - ppm) in fresh aubergines from Thailand	fruits and vegetables
2.	border rejection	13/11/2012	13/11/2012	2012.CGQ	FR	carbendazim (4.8 mg/kg - ppm) in aubergines from Thailand	fruits and vegetables
3.	information for attention	13/11/2012	13/11/2012	2012.1570	NL	methomyl (0.10 mg/kg - ppm) in green papaya from Thailand	fruits and vegetables
4.	border rejection	18/10/2012	07/11/2012	2012.CBB	GB	thiacloprid in fresh okra from India	fruits and vegetables
5.	border rejection	30/10/2012	30/10/2012	2012.CDM	NO	carbofuran (0.075 mg/kg - ppm) in chilled coriander leaves from Thailand	fruits and vegetables
6.	border rejection	11/10/2012	11/10/2012	2012.BZX	DK	dicofol (1.2 mg/kg - ppm) in fresh red chilli from Thailand	fruits and vegetables
7.	border rejection	08/08/2012	08/08/2012	2012.BQG	FR	indoxacarb (0.047 mg/kg - ppm) in asparagus bean from Thailand	fruits and vegetables
8.	information for attention	30/07/2012	30/07/2012	2012.1084	FR	carbendazim (4.2 mg/kg - ppm) in longan from Thailand	fruits and vegetables
9.	information for attention	19/07/2012	19/07/2012	2012.1020	NL	carbendazim (2.2 mg/kg - ppm) in durian from Thailand	fruits and vegetables
10.	border rejection	04/06/2012	04/06/2012	2012.BEE	FI	methomyl (0.074 mg/kg - ppm), omethoate and dimethoate (sum: 0.095 mg/kg - ppm) in and unauthorised genetically modified green papaya from Thailand	fruits and vegetables
11.	information for attention	23/05/2012	30/05/2012	2012.0698	CH	profenofos (266 µg/kg - ppb) and omethoate (87 µg/kg - ppb) in small eggplants from Thailand	fruits and vegetables
12.	border rejection	21/05/2012	29/05/2012	2012.BCE	DK	carbendazim (0,28 mg/kg - ppm) in chilli from Thailand	fruits and vegetables
13.	information for attention	22/05/2012	23/05/2012	2012.0694	CH	unauthorised substance dinotefuran (110 µg/kg - ppb) in chilled kale shoot from Thailand	fruits and vegetables
14.	information for attention	22/05/2012	22/05/2012	2012.0692	CH	unauthorised substance chlorfluazuron (30 µg/kg - ppb) in chilled kale from Thailand	fruits and vegetables
15.	information for attention	26/04/2012	04/05/2012	2012.0593	DE	omethoate (0.69 mg/kg - ppm) in okra (Abelmoschus esculentus) from Thailand	fruits and vegetables
16.	border rejection	25/04/2012	25/04/2012	2012.AXX	FR	dimethoate (0.17 mg/kg - ppm) in eggplants from Thailand	fruits and vegetables
17.	border rejection	07/03/2012	07/03/2012	2012.AOV	DE	dimethoate (0.91 mg/kg - ppm) in water mimosa from Thailand	fruits and vegetables
18.	border rejection	17/02/2012	23/02/2012	2012.ALN	DE	omethoate and dimethoate (sum: 1.039 mg/kg - ppm) in water mimosa from Thailand	fruits and vegetables

Question 2: Quantitation

- **Analysis by LC-MS/MS and GC-MS/MS**
  some targets can only be analyzed either by LC or GC.
- MS/MS mode: A parent ion (e.g. $[M+H]^+$) is selected in Q1, fragmented in Q2, and two specific daughter ions are monitored in Q3 (Selected Reaction Monitoring, **SRM**).
  ➔ exclusion of noise/matrix; high sensitivity, high selectivity
- Identification: a target is identified by its retention time, two specific mass transitions (SRMs), and their relative intensity.
- Calibration of analytes and internal standards.
- Several hundreds of compounds can be monitored (**MRM**: multiple reaction monitoring)

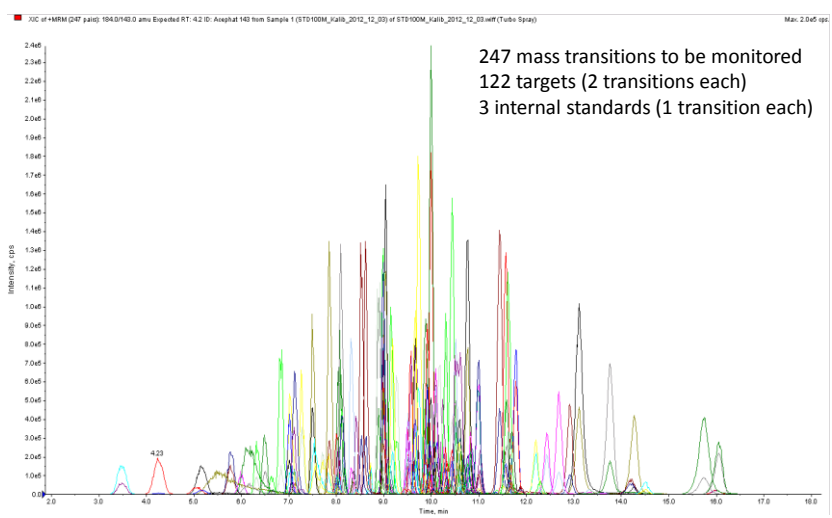




Question 2: Quantitation

- **IDEAL**: Use isotope-labeled standards for each compound
  - correction for losses during clean-up
  - correction for suppression effects in MS
  - very high costs (some targets are only rarely detected)
  - labeled standards are **not** available for all pesticides
  - more mass-transitions have to be monitored (400 → 800 MRMs)
    → dwell time per transition has to be lowered
    or number of data points in peak becomes (too) low
  - ➔ not efficient, not feasible
- **REAL:** Use several internal standards (e.g. 3) that elute at different times during chromatography
  - some correction for losses during clean-up
  - cheap and efficient
  - limited number of extra mass-transitions
  - no reliable correction for suppression effects
  - ➔ feasible
  - ➔ quantitation via standard addition for more accurate results

Question 2: Quantitation



Question 3: Confirmation

Options:

- Second analysis from the very beginning
- Change chromatography:
  LC ↔ GC, other stationary phase
- Change detector, e.g. from MS/MS to ToF with accurate mass
- Monitor more mass transitions and compare to standard
- Spiking experiment: recovery? Peak shape unchanged?
- Standard addition for more accurate quantitation
  ➔ matrix effects are compensated for

Plausibility of residue: does the result make sense?

Question 3: Further investigation

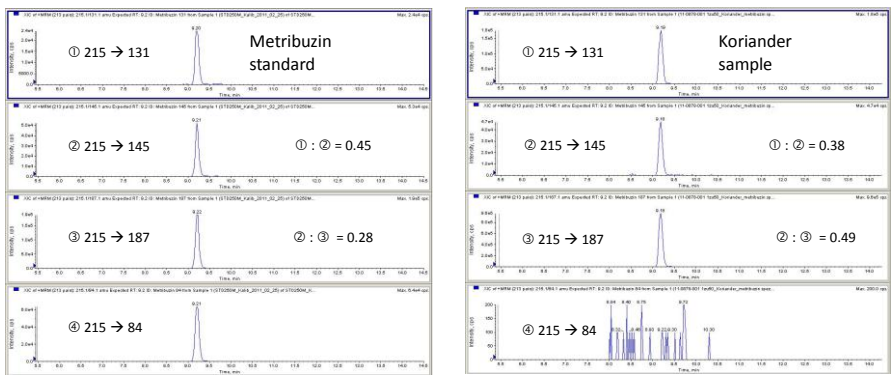
- Is a legal limit exceeded?
  with/withou measurement uncertainty?
- Is the substance prohibited (e.g. DDT)?
- Is the result not plausible?
- Is the sample especially important,
  e.g. organic?
- Is there health concern?
- Is the application of the pesticide illegal on the present sample?
- Is the sample analysed not fully homogenous?
- Can a mistake in samples not be excluded?

one YES! is enough to make further investigation!

False positives and false negatives must be avoided! Which one is worse?

Question 3: Example

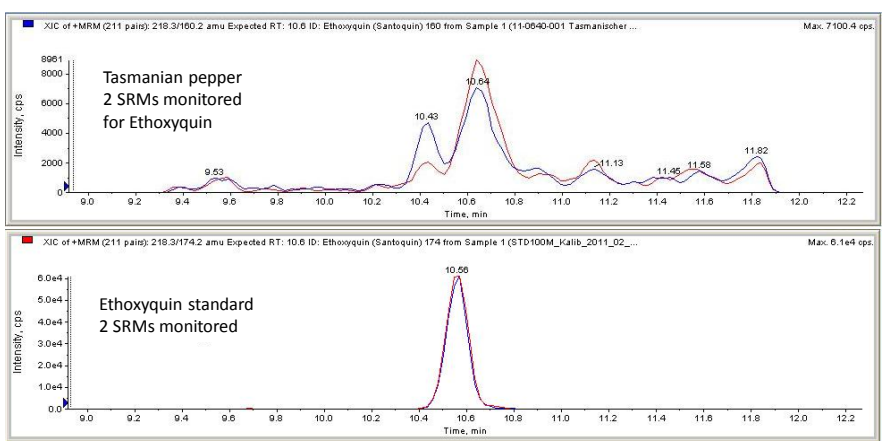
Example: GC-MS/MS Analysis indicates Buprofezin in Koriander.
Sample is analysed by LC-MS/MS using 4 MRMs.



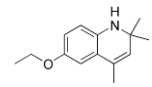
➔ Only the 4th mass transition reveals that the result is false positive!
➔ Intensities of the transitions differ between standard and sample (② : ③!)

The nasty case…

Tasmanian pepper with ethoxyquin?!
Concentration above MRL! False positive?

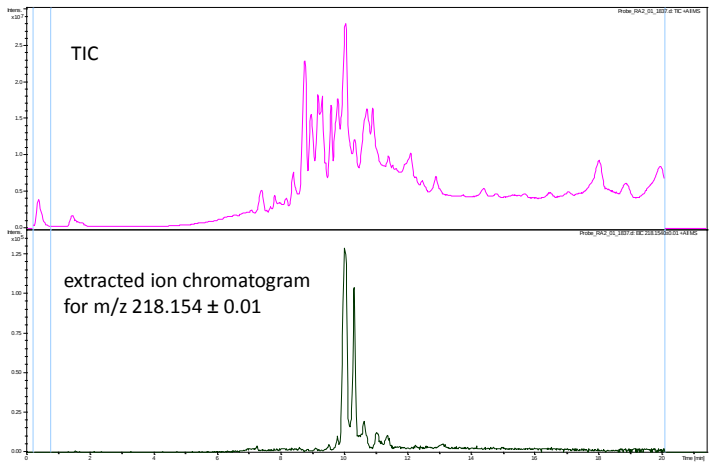


Tasmanian pepper



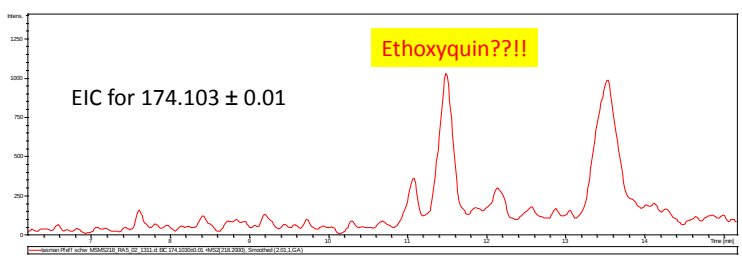
Ethoxyquin

Fullscan measurement with LC-Q-ToF
Ethoxyquin: exact mass of $[M+H]^+$ 218.1539



Tasmanian pepper

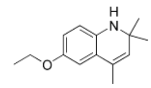
MS-MS experiment with LC-Q-ToF
Fragments of m/z 218

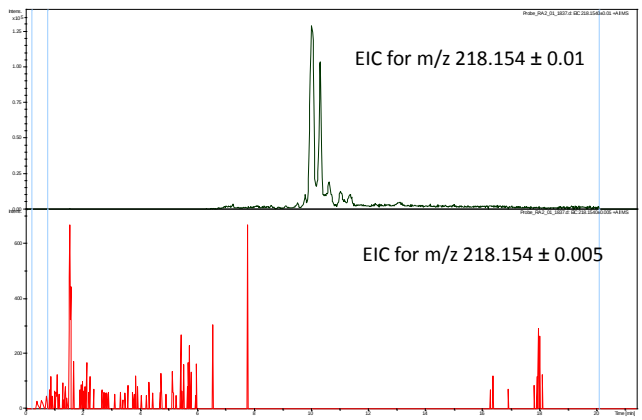


Some shifts of retention time are observed but are not unusual with spices.
Is it really positive? If yes ➔ no purchase!

Tasmanian pepper

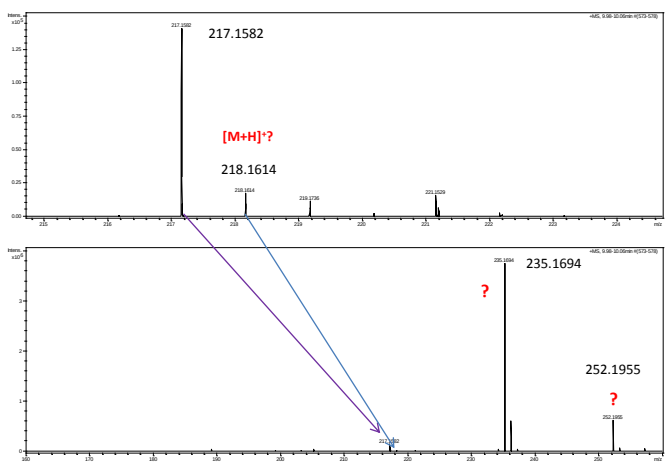
Fullscan measurement with LC-Q-ToF
Ethoxyquin: exact mass = 218.1539
Extracted ion chromatograms (EIC)



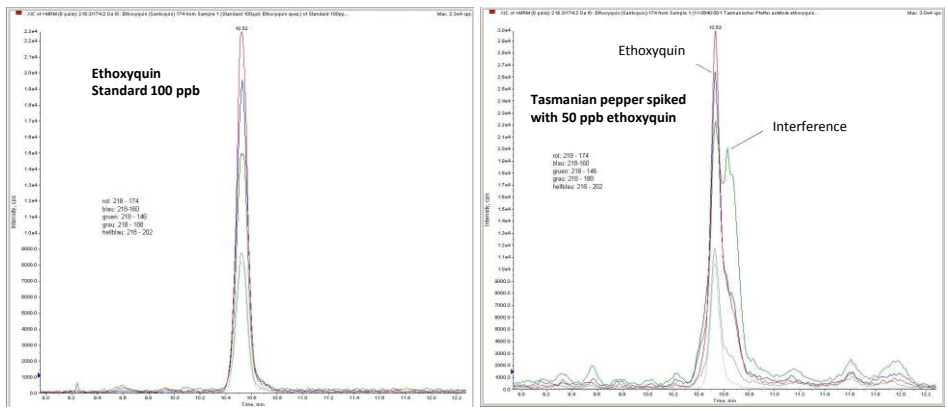


Tasmanian pepper

Fullscan measurement with LC-Q-ToF
Mass spectra of the suspected Ethoxyquin-peak

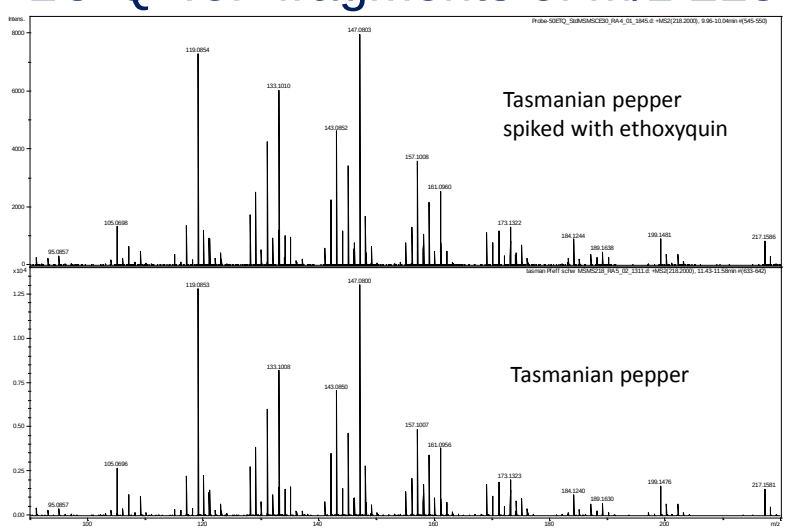


Tasmanian pepper: more SRMs and spiking



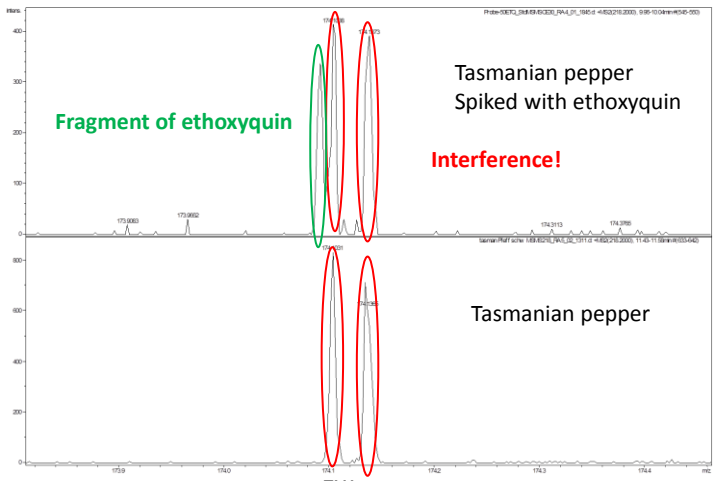
There is an interference showing all 5 (!) mass transitions (SRMs) ….
… but is there also some real ethoxyquin?

Tasmanian pepper: LC-Q-ToF fragments of m/z 218

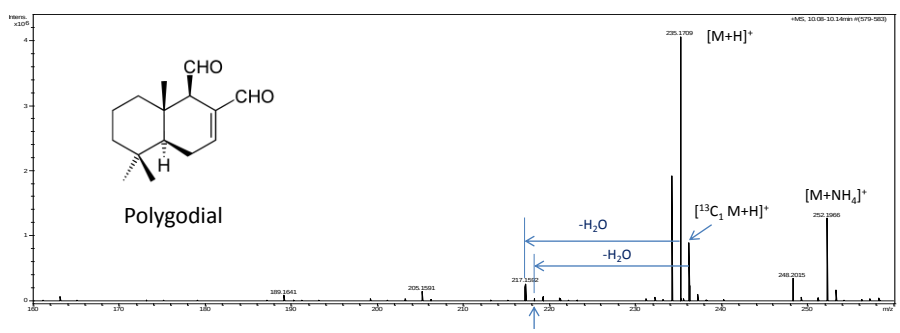


Tasmanian pepper: finally a closer look: no ethoxyquin!!

LC-Q-ToF: fragments of m/z 218



Tasmanian pepper: but what is it?



Polygodial is a natural component of tasmanian pepper
Exact mass: $[M+H]^+ = 235.1693$
During ionization polygodial undergoes a spontaneous loss of water
Its ^{13}C isotope thereby generates m/z 218.1621 (ethoxyquin: 218.1539)
This ion produces virtually the same fragment spectrum as ethoxyquin!

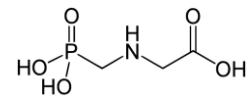
Tasmanian pepper: finally, we sell it!

- Polygodial is a natural component of tasmanian pepper, concentration is in the %-range.
- Polygodial can simulate the presence of ethoxyquin.
- Falso positive results can be avoided by careful investigations using triple quad and/or Q-ToF technologies.
- A false positive result whould have stopped the purchase
- Polygodial causes a numbing / tingling sensation on the tongue and thereby contributes an important part to the product characteristics.

Question 4: potential problems in retro-analysis

- Was the compound of interest covered by the sample preparation? If not ➔ risk of false-negatives!
- Quantitation: is the response of the detector well comparable between actual and former measurement?
  ➔ risk of over-/under-estimation
- If no reference standard is available and information on retention time is lacking: how can a "positive hit" be confirmed?
  ➔ risk of false-positives!
- Is the sample still available for confirmation analysis, including extraction?
- Is the analyte of interest stable in the sample?

Question 5: Glyphosate?



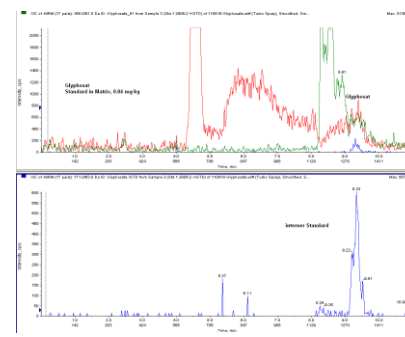
- Non-selective, systemic herbicide; Number 1 pesticide in the world! "kills everyhting green"
- First marketed in 1974 by Monsanto: RoundUp
- RoundUp Ready Soja: GMO-Soy from Monsanto, resistant to glyphosate
- Inzwischen gibt es zahlreiche Anbieter von Glyphosat-haltigen Herbiziden, z.B. Syngenta, Dow, etc.
- Application:
  - viticulture
  - fruits (e.g. blackberries, apples, …)
  - against weeds on uncultivated landder Brache
  - roadsides
  - siccation before harvesting (lentils, wheat, soy, …)

Question 5: how to analyse glyphosate?

Option 1:

- Aqueous extract
- Anion exchange chromatography
- MS/MS detection
- Isotope labeled internal standards

- simple, cheap, fast
- a lot of co-extractives
  ➔ some matrices ruin column with 5 injections
- LOQ may be too high (0.01 mg/kg is the goal)



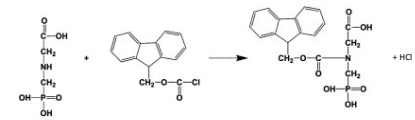
Question 5: how to analyse glyphosate?

Option 2:

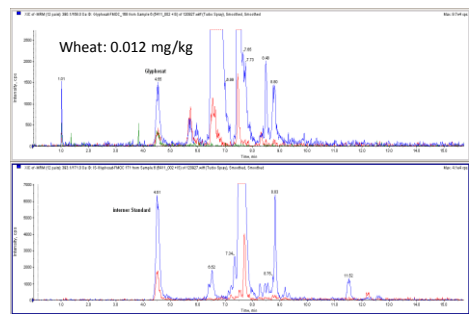
- Aqueous extract
- Derivatisation with FMOC-Cl
- Derivate is much less polar and can be enriched on a SPE-cartridge
- Classical C18-LC-MS/MS works fine
- Isotope labeled internal standards

- rather clean extracts
- low LOQ can be achieved (0.01 mg/kg)
- more expensive (time, chemicals, work)

➔ Method of choice!



Glyphosate FMOC-Cl Glyphosate-FMOC Derivative



Question 7: Assessment of residues

- FIV: Fremd- und Inhaltsstoffverordnung
  http://www.admin.ch/ch/d/sr/8/817.021.23.de.pdf
  Swiss Maximum Residue Levels (MRLs)
- European MRLs:
  http://ec.europa.eu/sanco_pesticides/public
- Swiss pesticide database:
  www.blw.admin.ch/psm/produkte/index.html?lang=de
- Pesticides allowed for application on fruit for "Suisse garantie"
  www.swissfruit.ch/m/mandanten/239/download/2012_Saio_wirkstoffe_liste_d_komplett.pdf

Question 7:
Assessment of residues

Pesticide	Conentration mg/kg	Horwitz mg/kg	MRL mg/kg	Application allowed on blackberries?	Complaint?
Buprofezin	0.09	0.021	0.1	yes	no
Bifenthrin	0.67	0.11	0.3	No!	Yes!
Spinosad	0.02	0.0057	0.5	No!	Yes!
Folpet	2.24	0.32	3	yes	no
Cyprodinil	0.01	0.0032	10	yes	no

Thank you for your attention!

Acknowledgement to Thomas Döring
for LC-Q-ToF measurements and data