

FPRW 2010

Forum on Mass Spectrometry

Debating different approaches to chemical residue identification using MS



Two Approaches to MS ID

- 1) Commission Decision concerning the performance of analytical methods and the interpretation of results. (EU/657/2002)

Also: *Tr. Anal. Chem.* (2001) **20**, 435.

Dr. Jean-Philippe Antignac

National Veterinary University of Nantes, France

- 2) Method validation and quality control procedures for pesticide residues analysis in food and feed. (SANCO/10684/2009)

Also: *Tr. Anal. Chem.* (2008) **27**, 1070.

Dr. Hans Mol

RIKILT, Wageningen, The Netherlands



Pesticides: context

EU pesticide legislation (MRLs)

Non registered pesticides:

Not registered in EU (banned, never notified)

Not registered for a certain crop

Registered pesticides:

GAP: no detectable residue at harvest

residue tolerance established



MRL = 0.01 – 10 mg/kg*

(some exceptions: 0.002-0.006 mg/kg)

MRL = LOQ

by default 0.01 mg/kg

* Most cases: MRL well below toxicologically relevant levels

Pesticides: context

What's at stake?

Enforcement and monitoring (governmental labs)

Purpose of analysis:

Enforcement of MRLs (consumer products)

false positive: incorrect rapid alert, economic loss, erroneous fine, law suit, loss of confidence of enforcement lab

false negative: unnecessary exposure of consumer to enhanced levels of chemicals; intoxication (rare)

Enforcement of use of registered pesticides (pre-harvest food; non-food)

false positive: erroneous fine, law suit (rare), loss of confidence in lab

false negative: unnoticed use of illegal pesticides, worker exposure/env. issues

Levels below MRL: data for risk assessment / trend analysis

false positive: incorrect risk assessment (overestimation of exposure)

false negative: incorrect risk assessment (underestimation of exposure)

Contract labs

Purpose of analysis:

Analytical services to food industry/retail/etc (make money)

false positives/false negatives: lab reputation, loss of contracts

Pesticides: EU guidance document

**METHOD VALIDATION AND
QUALITY CONTROL PROCEDURES
FOR
PESTICIDE RESIDUES ANALYSIS IN
FOOD AND FEED**

Document No. SANCO/10684/2009

Supersedes Document No. SANCO/3131/2007

Implemented by 01/01/2010

Guidance document for laboratories involved in official monitoring and control of pesticide residues in food and feed

Revised/updated every 2 year

http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf

Pesticides: brief history

Initiated in 1997 by Alan Hill (UK), Andre de Kok (NL), Arne Anderson (S) to harmonise AQC for pesticide residue analysis in food

Identification: early version: GC-MS (3 ion criterion)

2006: criteria for MS/MS

Considered: experience, literature, other criteria documents

⇒ don't re-invent wheel,

⇒ within EU: try to be consistent

2002/657 rt and ion ratio criteria (3 for GC-MS, 2 transitions for MS/MS)

Incorporated into pesticides AQC guideline

But: use as guideline (no scientific rational; enormous variation pest/matrix)

ID point system not incorporated (no scientific rational)

Update of SANCO/3131/2007 in 2009:

Incorporation of high res MS

Removing inconsistencies for interpretation GC-MS full scan vs SIM

Pesticides: identification

72 Use of selective detectors (ECD, FPD, NPD, DAD, Flu):
Not suited for unambiguous identification

74 Chromatography

$$t_r \geq 2 \cdot t_0$$

Tolerance for relative retention time: GC $\pm 0.5\%$ LC: $\pm 2.5\%$

76 Selection of diagnostic ions

- Include (quasi) molecular ion if possible
- Avoid $m/z < 100$ and product ions $-H_2O$ or $-NH_3$ if possible
- Cl, Br isotopes can be used but diagnostic ions should not exclusively originate from same part of molecule
- Choice of diagnostic ions may change depending on matrix
- $S/N \geq 3$

79. Different types and modes of mass spectrometric detectors provide different degrees of selectivity, which relates to the confidence in identification. The requirements for identification are given in Table 3. They should be regarded as guidance criteria for identification, not as absolute criteria to prove presence or absence of a compound.

Pesticides: identification criteria

Table 3 Identification requirements for different types of mass spectrometers

MS mode:	Single MS (standard mass resolution)	Single MS (high resolution/high mass accuracy)	MS/MS
Typical systems (examples)	quadrupole, ion trap, time-of-flight (TOF)	TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole ion trap, hybride MS (e.g. Q-TOF, Q-trap)
Acquisition:	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM), full scan product-ion spectra
Requirements for identification:	≥ 3 diagnostic ions, (preferably including quasi molecular ion)	≥ 2 diagnostic ions (preferably including the quasi molecular ion). Mass accuracy < 5 ppm. At least one fragment ion.	≥ 2 product ions
In 2002/657 ID points:	3 points	4 points	4 points
Ion ratio(s):	according to Table 4		

Pesticides: identification criteria

Table 4. Default recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques².

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS _n , LC-MS, LC-MS _n (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10%	± 50 %	± 50 %


Ratio measured at same conditions, similar concentration, solvent or matrix matched

Actual measurement of the variability of the ion ratios can be conducted experimentally over time using calibration standards to devise performance-based criteria rather than the fixed generic criteria given in Table

As for Table 4, the tolerances should not be taken as absolute limits and automated data interpretation based on the criteria without complementary interpretation by an experienced analyst is not recommended.

Pesticides: identification criteria

Depending on purpose of analysis / what's at stake

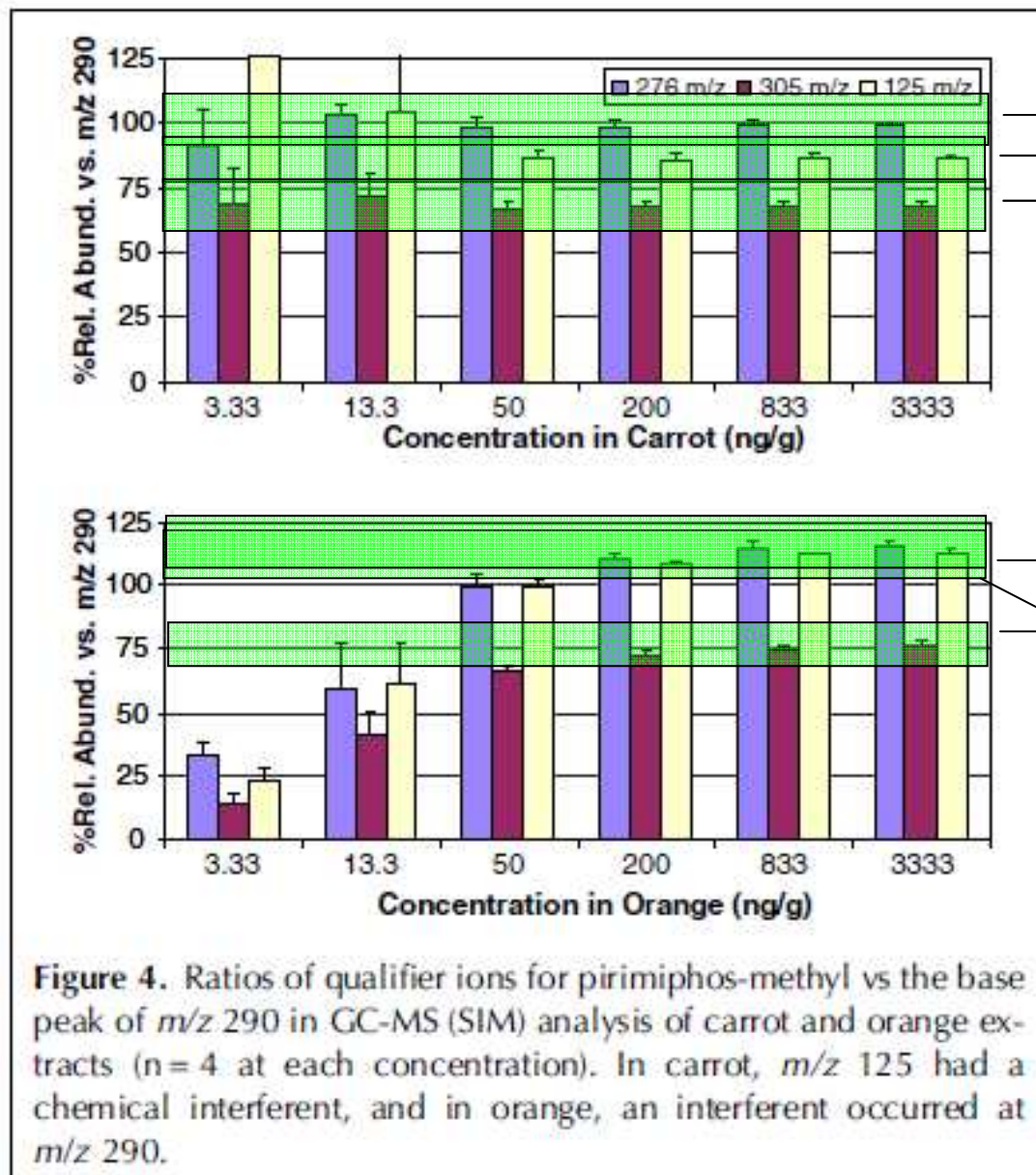


For a higher degree of confidence in identification, further evidence may be required. This can be achieved through additional mass spectrometric information, for example evaluation of full scan spectra, additional accurate mass (fragment) ions, additional product ions (in MS/MS), or accurate mass product ions. If the isotope ratio of the ion(s), or the chromatographic profile of isomers of the analyte, is highly characteristic it may provide sufficient evidence. Otherwise, additional evidence may be sought using a different chromatographic separation system and/or a different ionisation technique, or any other means providing supporting information.

Occurrence of false negatives and false positives using SANCO/10684

Real World Examples

Evaluation false negatives



GC-MS (SIM)

276 vs 290 OK down to 3.33
125 vs 290 OK down to 50 (interfered)
 305 vs 290 OK down to 3.33

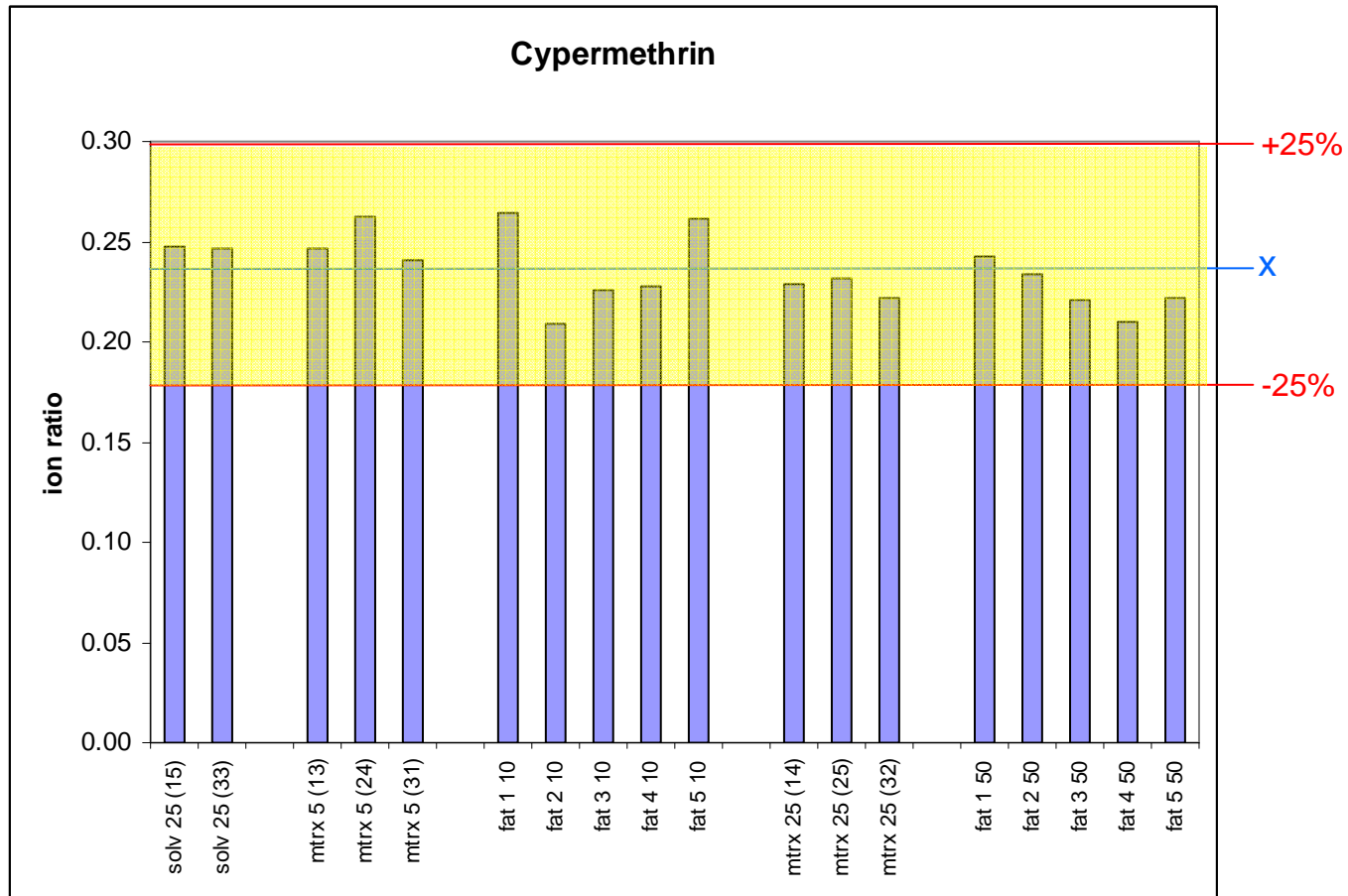
276 vs 290 OK down to 200
125 vs 290 OK down to 200
305 vs 290 OK down to 200

290 interfered

⇒ Ratio: matrix, ion, conc. dependent
 ⇒ Selection of diagnostic ion
 matrix dependent

Evaluation false negatives

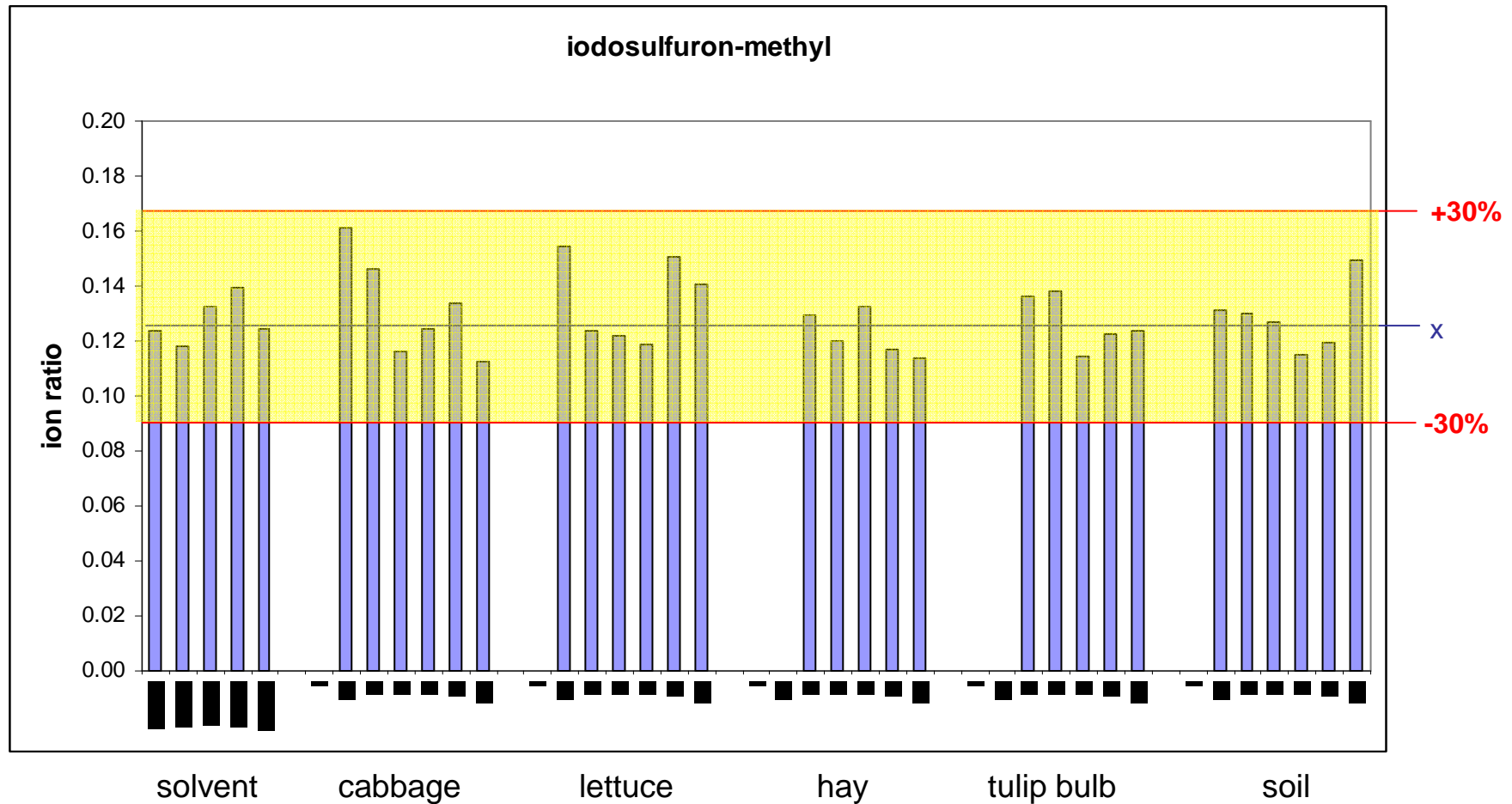
GC-MS/MS spiked fat samples from 5 species, 10 and 50 µg/kg



Performance compliant with criteria, irrespective matrix/concentration

Evaluation false negatives

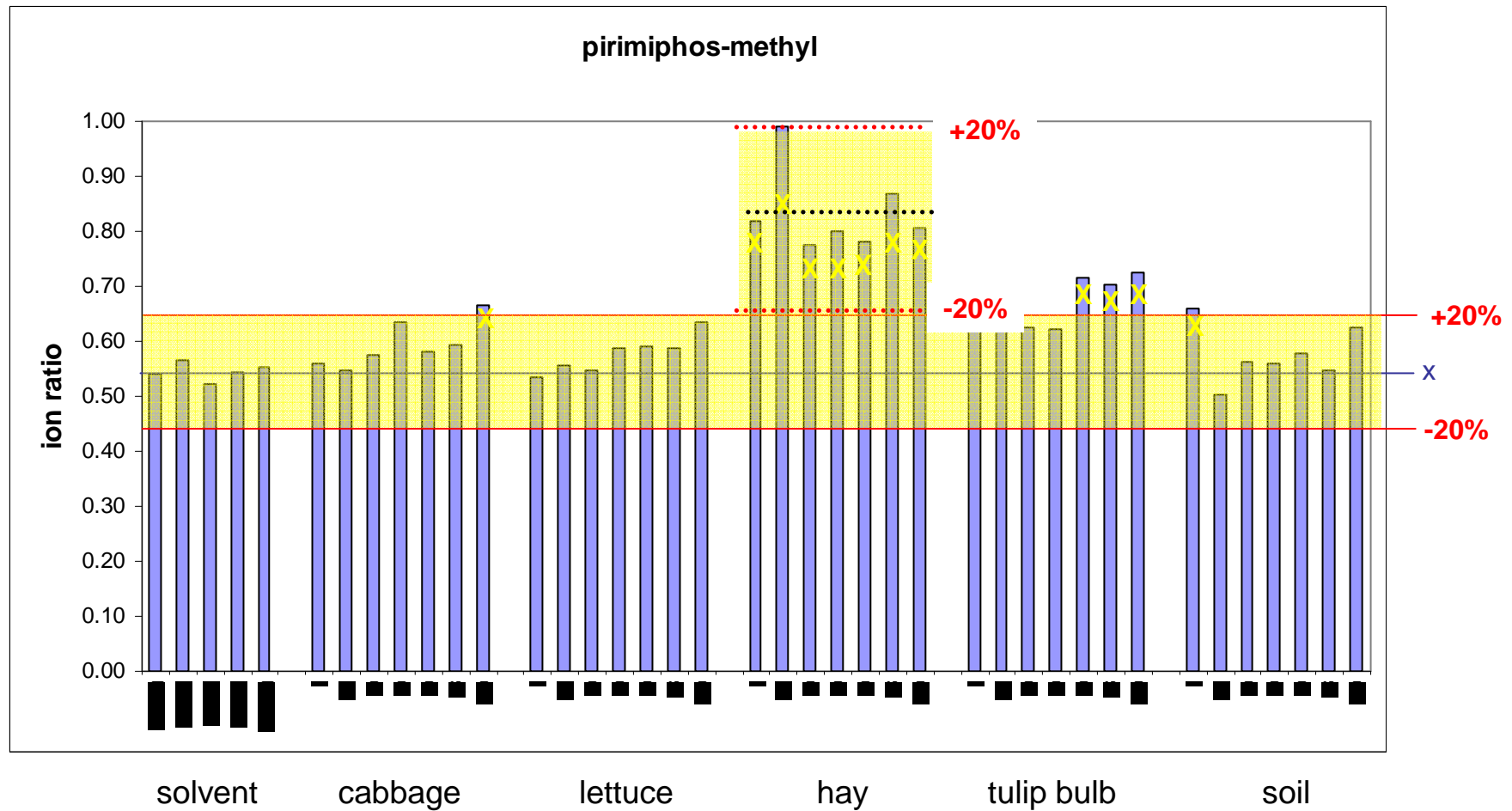
LC-MS/MS from validation sequence (measurement on same day)
Thresholds based on average ratio of solvent standards injected
in between the samples



Performance compliant with ion ratio criterion, irrespective the matrix

Evaluation false negatives

LC-MS/MS from validation sequence (measurement on same day)
Thresholds based on average ratio of solvent standards injected
in between the samples



Compliance with ion ratio criterion matrix dependent

Evaluation false negatives

LC-MS/MS

Organophosphorus pesticides and carbamates (45 analytes, 2 transitions

Milk (cow), meat (5 species), liver (5 species, duplicate)

2 levels: 5 and 25 µg/kg

Sequence: 79 injections solvent std, matrix standard, spiked samples

3 compounds: no 2nd transition at lower level (data points excluded)

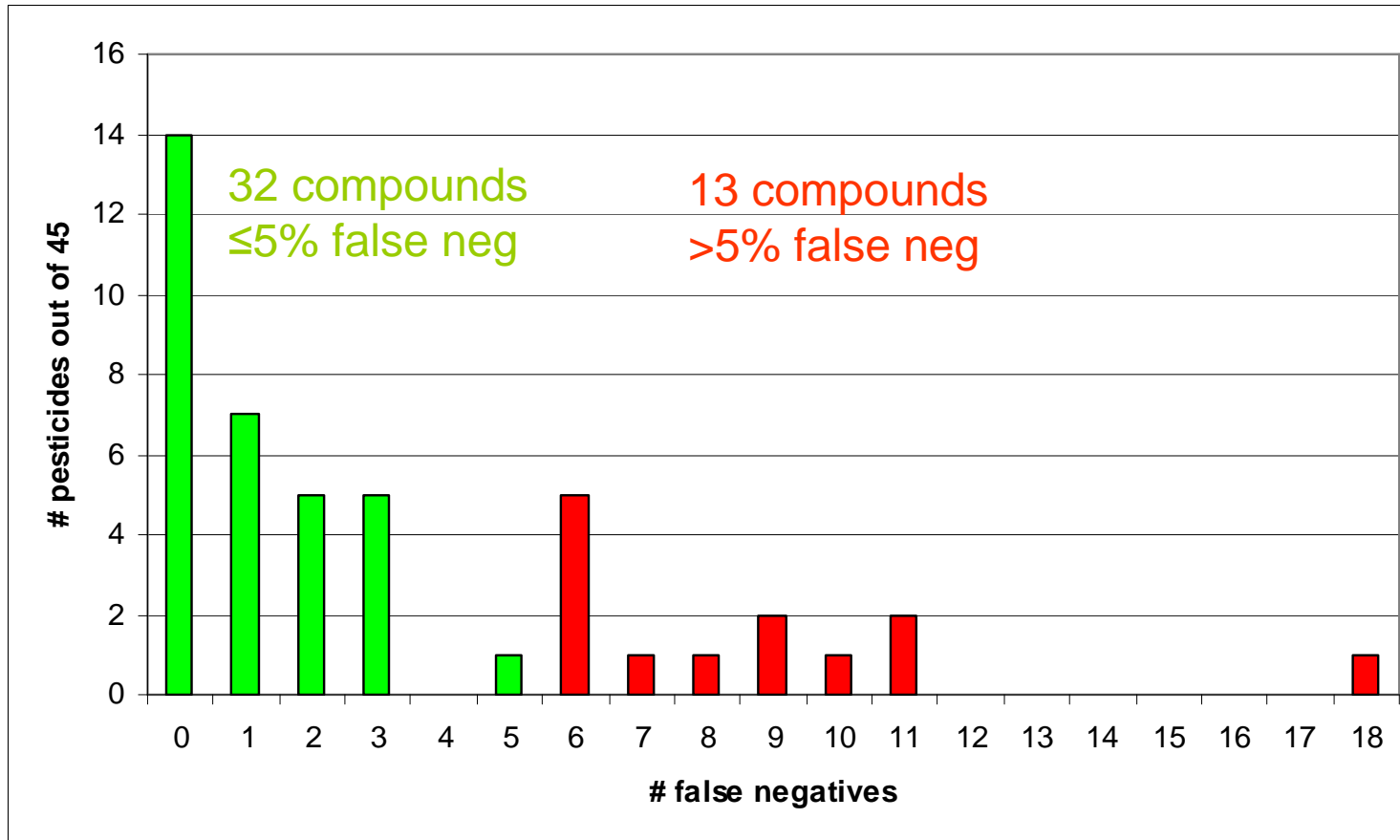
Total # ratios considered: 3349

Overall false neg rate

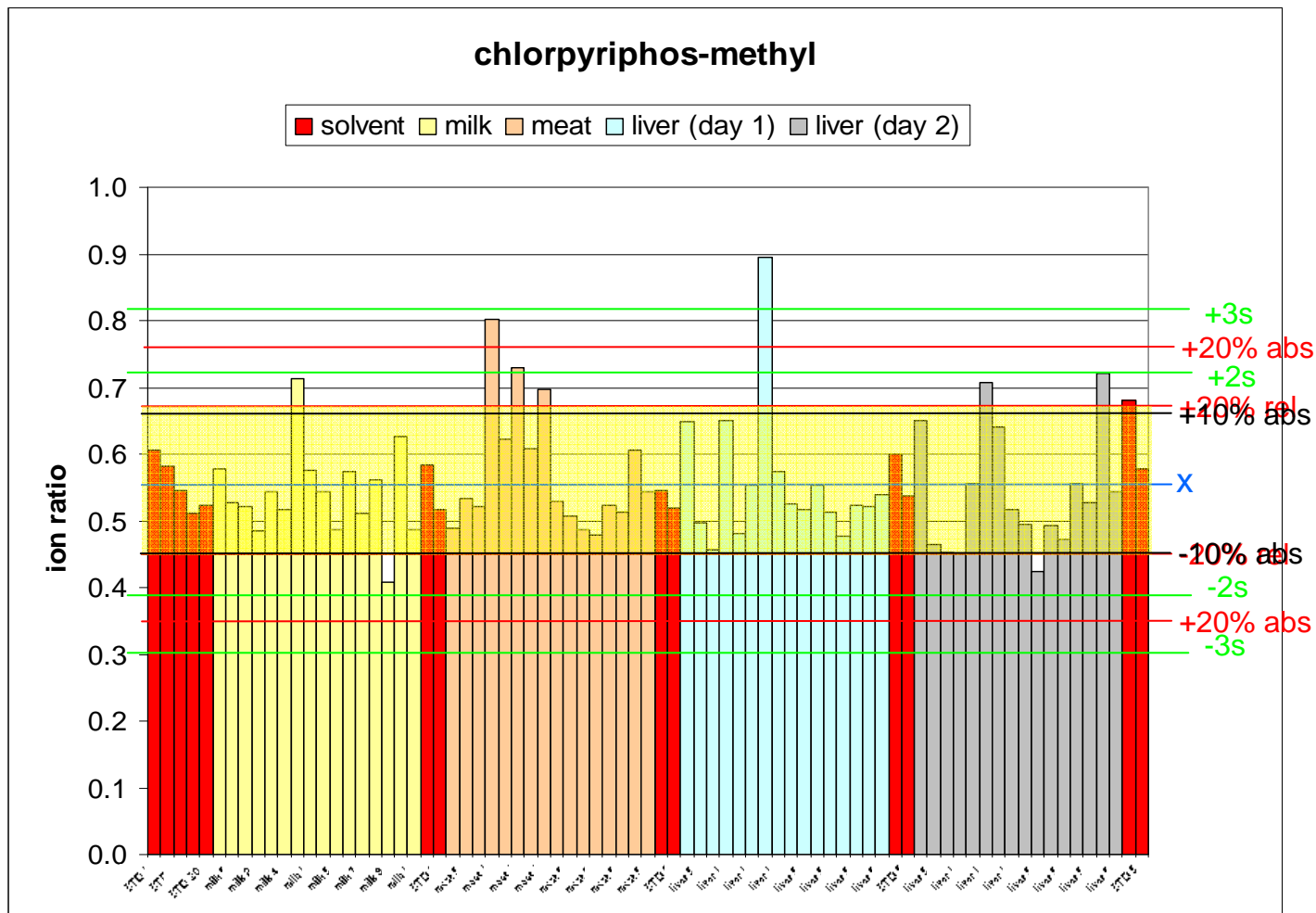
	pesticides present but not meeting ion ratio criterion	
Criterion	number	%
Sanco/10864	150	4.5
± 2SD	161	4.8
± 3SD	31	0.9

Evaluation false negatives

But at an analyte level:



Evaluation false negatives



False neg rate according to various criteria:

20% rel. 10 (13%) [EU 2002/657 and sanco/10684/2009; US FDA ORA-LAB.10]

20% abs. 2 (2.5%) [USDA PDP-DATA 04/15/10]

10% abs. 9 (11%) [US FDA/CVM Guidance #118; WADA TD2010IDCR]

Based on actual variability: 2SD: 4 (5%); 3SD: 1 (1.2%)

Evaluation false positives

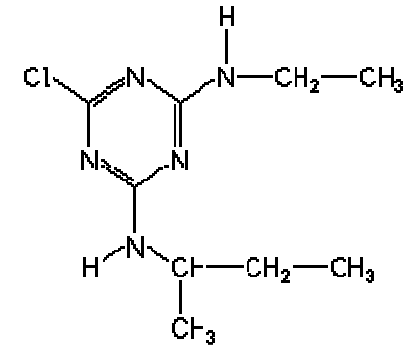
General experience using SANCO/10684/2009 guideline (\approx 657/2002):
1000's samples analyzed for 100s compounds using various methods
 \Rightarrow Only very low numbers of findings meeting criteria
 \Rightarrow criteria (and methods used) seem adequate to avoid false positives

Own experience: false positives occur but are almost exclusively due to

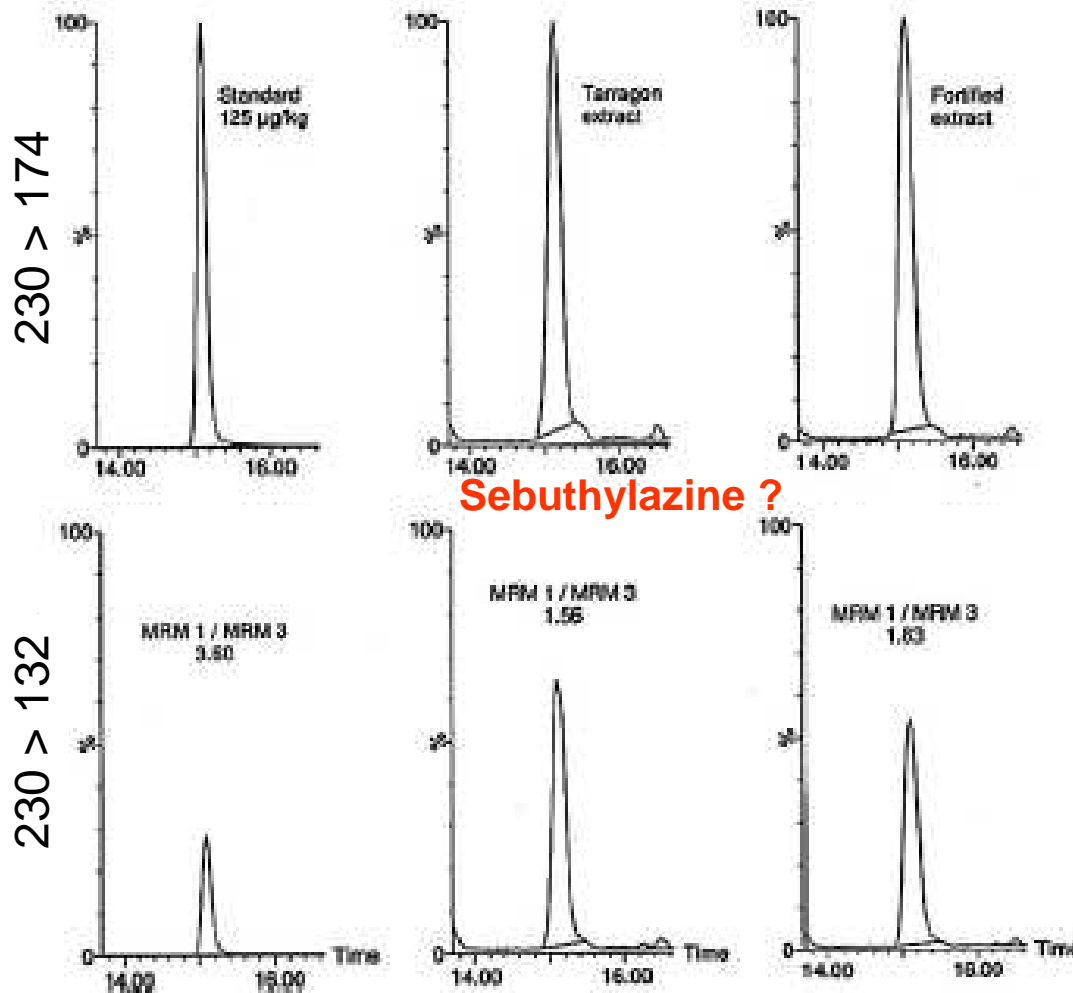
- exchange/coding of samples/vials/etc
- cross contamination/carry over
- trivial errors: copy/past, reporting

But of course there are exceptions.....

Evaluation false positives



Schurmann et al, Rapid Comm. Mass Spectr. 23 (2009) 1196
 Kaufmann et al, Anal. Chim. Acta xx (2010) in press



LC-MS/MS

2 transitions, ratio criterion met

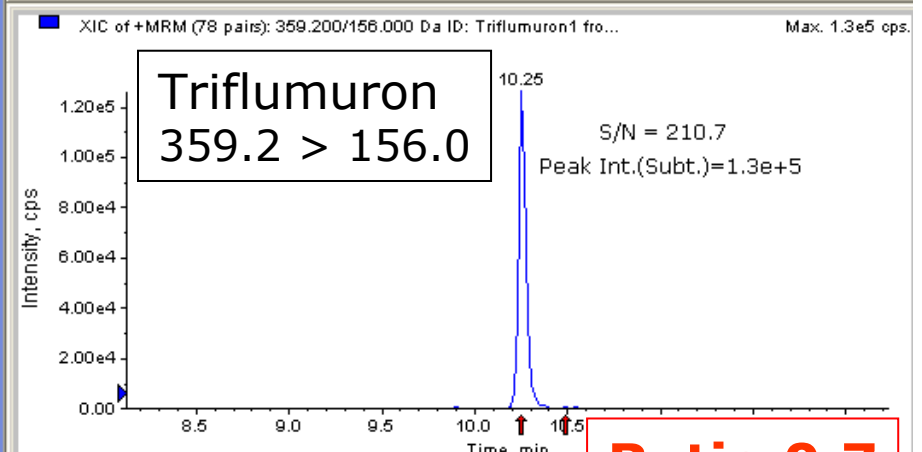
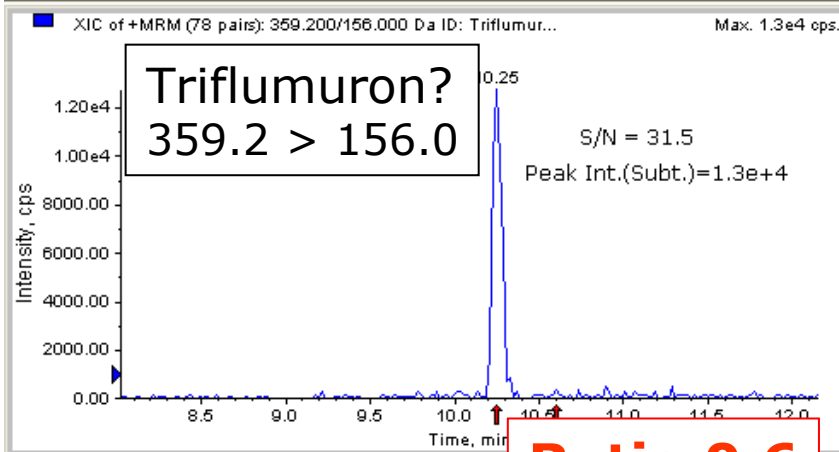
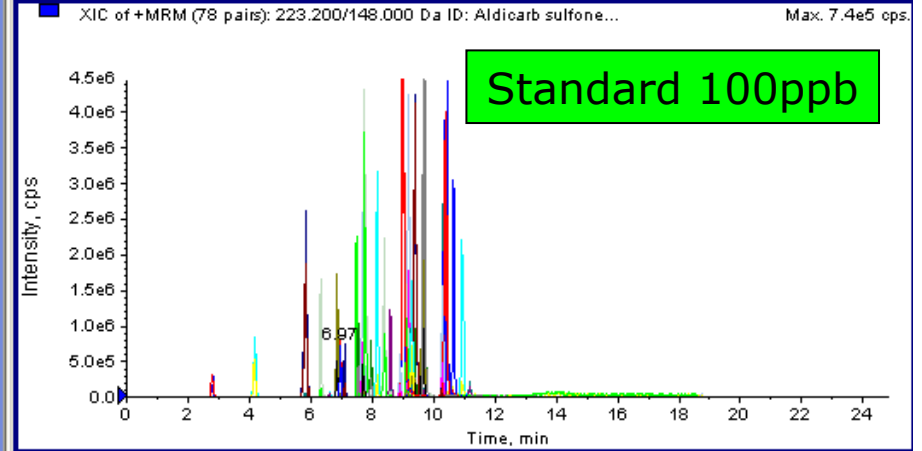
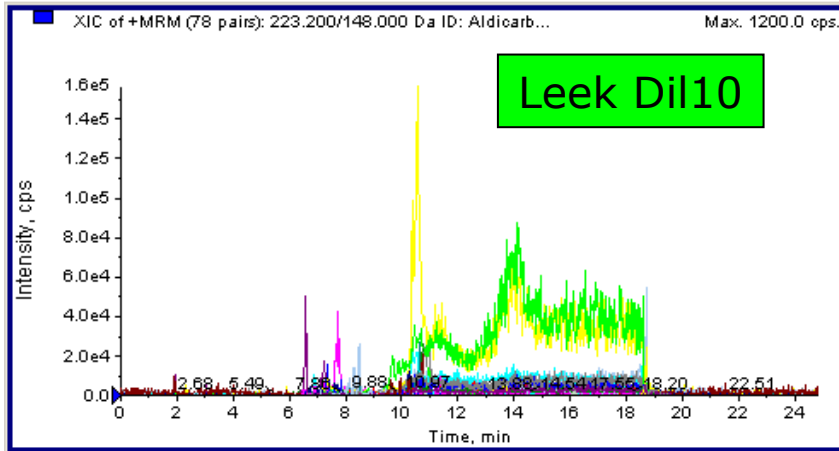
Confirmation by GC-MS/MS

Both transitions absent in sample
 (present in fortified sample)

3rd transition in LC-MS/MS

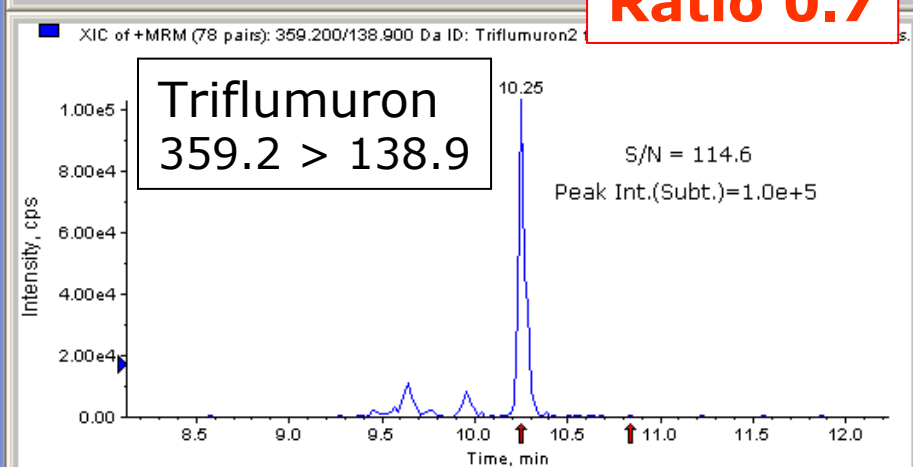
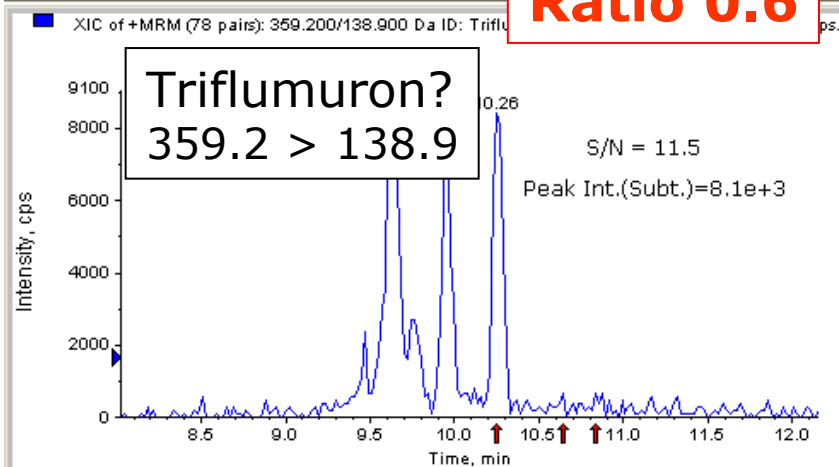
ratio deviating from solv. standard

Source: Amadeo Fernandez-Alba, University Almeria, Spain (EURL)



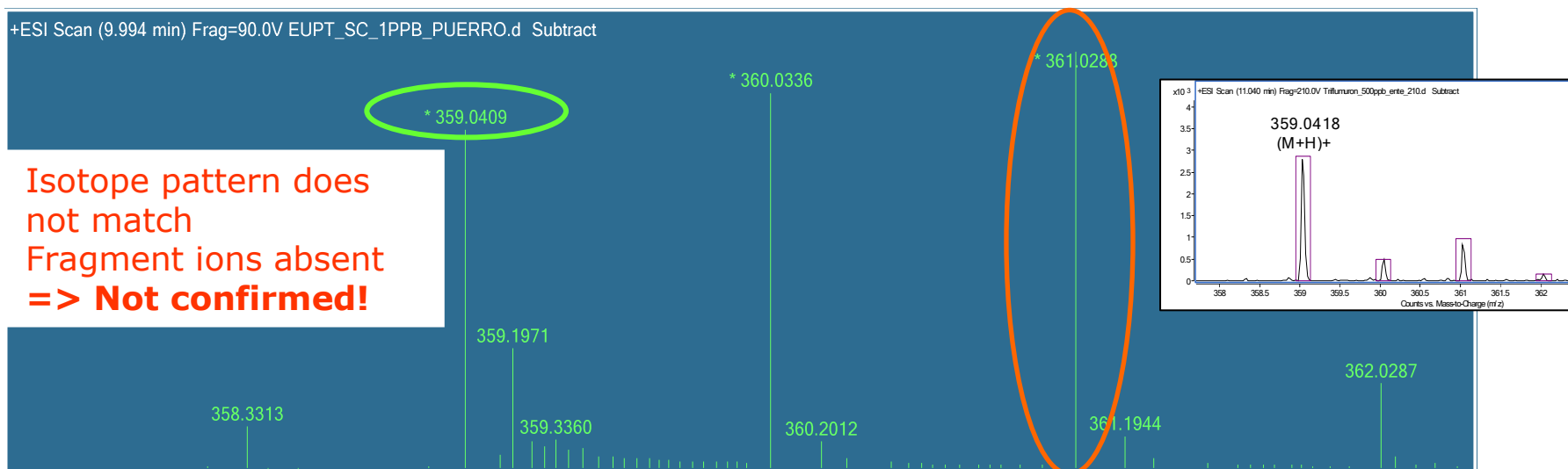
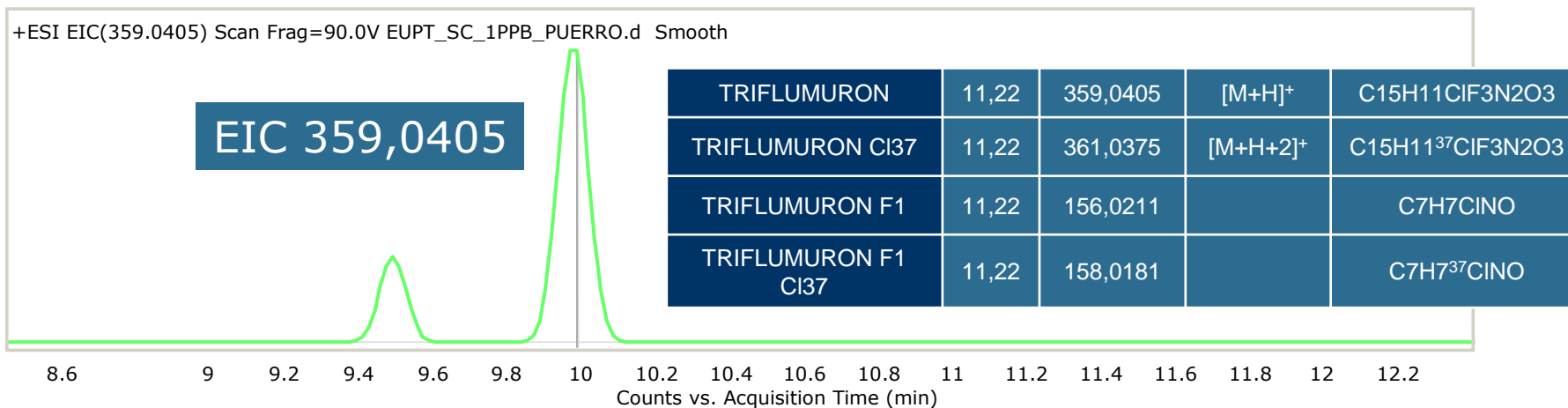
Ratio 0.6

Ratio 0.7



Evaluation false positives

Leek sample: confirmatory analysis by LC-TOF-MS



Closing remarks

- EU criteria for pesticides not very different from vet drugs
main difference: guidance vs absolute criteria/law
- Too many variables for fixed identification criteria suited for all situations
- Current criteria lack solid scientific basis,
however: false positives are rare
On the other hand: false negatives: do occur, frequency depends on
analyte, concentration, matrix, sample prep, instrumental analysis
- During validation and on-going AQC there should be more emphasis on
evaluation of qualitative aspects (variability of identification parameters) to
tune criteria to set fit-for-purpose balance between false pos/false neg
- Identification should be based on scientific data combined with
expert judgment. The degree of confidence / effort put into the identification
should be related to purpose of analysis / consequences of the result.
Any finding with legal consequences should be confirmed by re-analysis
of the sample, preferably with an orthogonal technique.