Identification criteria for residues determined by LC-MS/MS: are they fit-for-purpose?

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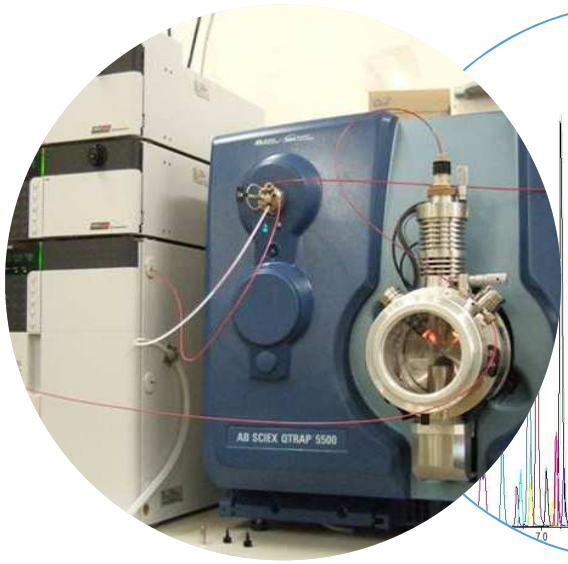

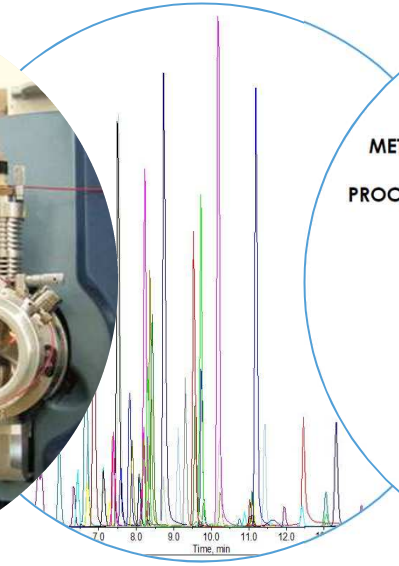

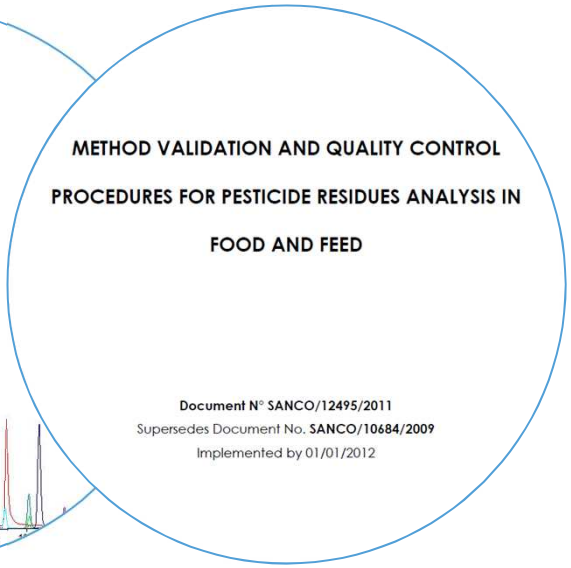

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EURL-Pesticides joint workshop
Almeria, 23-25 October 2013

European Commission
EURL-FV

Content

- Current criteria and their origin
- Criteria elsewhere
- Systematic assessment of experimental (R)RT and ion ratio deviations vs EU criteria for LC-MS/MS (triple quadrupole)
- Conclusions and impact on revision of AQC document
- Outlook to other LC-MS, GC-MS techniques

Further justification/clarification of proposed changes in draft AQC and discussion during AQC session

Identification of pesticide residues in EU

SANCO/12495/2011

73 Use of selective detectors (ECD, FPD, NPD, DAD, Flu):
Not suited for unambiguous identification
⇒ Chromatography with mass spectrometry is required

75 Chromatography

$t_r \geq 2*t_0$

Criterion for relative retention time: GC ±0.5% LC: ±2.5%

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Identification of pesticide residues in EU

76-80 Mass spectrometry

Requirement with respect to # diagnostic ions

Table 4. 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
Ion ratio(s):	according to Table 5		

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Identification of pesticide residues in EU

76-80 Mass spectrometry

Requirement with respect to ion ratio

Table 5. Default recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques[2].

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 %

Evolution of (LC-MS/MS) identification criteria

1997: pre-EURL era: initiative by A. Hill (UK), A. de Kok (NL), A. Anderson (S) to harmonise AQC for pesticide residue analysis in food
Identification: qualitative description, emphasis on GC-MS
EICs: similar RT, peak shape, response ratios
FS: intensity ratios of principle ions within 80-120% of standard
SIM: ≥2 ions m/z >200 or ≥3 ions m/z >100

S19, Luke, EtOAc
+ LLE, GPC, SPE
GC-ECD/NPD/FPD
GC-MSD, ITD
HPLC-UV; Flu

1999: FS: intensity ratios of principle ions should be within 70-130% of standard

2003: -

2006: Incorporation of RT and ion ratio criteria from CD 2002/657/EC
RRT should be within ±0.5% for GC and ±2.5% for LC
Ion ratio:

QuEChERS
GC-MSD, ITD
LC-MS/MS

Table 3. Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques [2]

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 %

QuEChERS
GC-MSD, ITD
GC-MS/MS
LC-MS/MS
(U)HPLC

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Evolution of (LC-MS/MS) identification criteria

2007: -

2009: Revision of text on identification
Different types of MS specifically addressed

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
Ion ratio(s):	according to Table 4		

2011: -

QuEChERS
mini-luke, SweEt, dil& shoot
(U)HPLC-MS/MS
TOF, Orbitrap
GC-MSD/ITD/TOF; GCxGC-TOF
GC-MS/MS

QuEChERS
mini-luke, SweEt, dilute&shoot
(U)HPLC-MS/MS
LC-(Q)TOF, (Q)Orbitrap
GC-MSD/ITD/TOF, GC-MS/MS,
GCxGC-TOF, GC-QTOF, GC-APCI-MS
FI-MS, DMS, ion mobility

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EU: SANCO/12495/2011 vs 2002/657/EC

2002/657/EC (products of animal origin):

- Fixed legislative rule instead of guidance
- Identification point system
  - 3 ID points required for vet. drugs and contaminants (incl. pest.)
  - 4 ID points required for anabolic and unauthorized substances

Table 4. 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
2002/657 ID points:	**3 points**	**4 points**	**4 points**

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Developement in criteria vs methods

Identification criteria for LC-MS/MS in EU:

Fundament = 2002/657 (but discussed and created in late 1990's)

.....did not change since.....

Meanwhile in pesticide residue analysis:

MRLs from 0.05-0.1 => default 0.01 mg/kg

LC-MS/MS main stream technique

5 generations of instruments + new vendors

HPLC => UHPLC

Sample preparation: more generic extraction + little/no clean up => dirtier extracts

Scope from 20-50 pesticides to 200 (400 transitions) in 15 min.

Do the criteria still make sense?

Are they still fit-for-purpose?

LC-MS/MS identification criteria elsewhere

Source	Applies to	Retention time	# ions/transitions	Ion ratio rel. int. (% of base peak)	Maximum tolerance
EU: 2002/657/EC SANCO/12495	 vet. drugs in animal products pesticides in food/feed	RRT ± 2.5%	≥ 2 product ions	>50% >20-50% >10-20% ≤10%	±20% (rel) ±25% (rel) ±30% (rel) ±50% (rel)
FDA ORA-LAB.10	pesticides in food	RT ± experim. SD	≥ 2 product ions	>40% >10-40% ≤10%	±20% (rel) ±25% (rel) ±50% (rel)
USDA PDP Data	pesticides in food	RT ± 0.5 min RRT ± 0.1 min	≥ 2 product ions	all	±20% (abs)
FDA GfI 118	animal drug residues	RT ± 5%	≥ 2 product ions	2 diagn. ions ≥3 diagn. Ions	±10% (abs) ±20% (abs)
WADA	sports doping	RT ± 2% or 0.1 min RRT ±1% RRT ± 0.1% (isotopic ISTD)	≥ 2 product ions	>50% >25-50% >5-25% ≤5%	±10% (abs) ±20% (rel) ±5% (abs) ±50% (rel)
AORC	drugs in racing animals	RRT ± 2.5% RT ±2% or 12 s RT ±50%PWHH or 3 s	≥ 3 ions	all	±10% (abs) or ±30% (rel)
EWDTS	drugs in urine (workplace testing)	RT ± 2% or 3 s	≥ 3 ions	all	±20% (rel)
UNODC	illicit drugs (seized materials, biological specimens)	RT ± 2%	≥ 3 ions/transitions	all	±20% (rel)
SOFT / AAFS	forensic toxicology		≥ 2 ions	all	±25-30% (rel)

Multi-lab assessment identification

Objectives:

- Gain insight in actual variability of ion ratios and retention times obtained with today's generic sample prep & instrumentation under routine conditions
- Systematic investigation of variability of retention times and ion ratios
- To verify whether there (still) is a rational for the current EU identification criteria
- To provide experimental evidence to underpin future guidance on identification

Parameters affecting qualitative performance

- the pesticide: ions chosen, # ions, absolute response, relative abundance
- concentration => absolute response
- matrix: solvent, commodity, sample preparation, matrix equivalent introduced
- analysis technique (GC or LC, ionization mode, single MS; MS/MS; QqQ, QTOF)
- instrument and acquisition parameters used
- acquisition: dwell time; # datapoints across the peak
- stability of response of the instrument
- co-elution with other compounds

Multi-lab assessment identification

Too many options to cover all.....

⇒ Start with:

Technique:	LC-MS/MS (triple) [preferred technique for majority of pesticides]
Matrix:	Fruits & Vegetables
	[most frequently analysed + high incidence of residues]
Scope:	wide variety of pesticides in multi-method, different conc.

- Cover multiple laboratories = LC-MS/MS instruments/conditions
  5 labs: UA, CVUA, FERA, NVWA, RIKILT

Set up of project

- Wide variety of pesticides: classes, polarity, MW, sensitivity

abamectine	chlorpropham	etofenprox	kresoxim-methyl	oxamyl	pyrimethanil
acephate	chlorpyrifos	famoxadone	linuron	paclobutrazole	pyriproxyfen
acetamiprid	chlorpyrifos-methyl	fenamidone	lufenuron	parathion	spinosyn A
aclonifen	clofentezine	fenamiphos	malathion	parathion-methyl	spinosyn D
aldicarb	cyanazine	fenhexamid	metaflumizone	pencycuron	spiromesifen
aldicarbsulfon	cyazofamid	fenoxycarb	metalaxyl	pendimethalin	spiroxamine
aldicarbsulfoxide	cypermethrin	fenpropimorph	methamidophos	phorate	tebuconazole
azoxystrobin	deltamethrin	fenpyroximate	methidathion	phosmet	tebufenozide
bifenazate	desmedipham	fenthion	methiocarbsulfon	phosphamidon	tebufenpyrad
bifenthrin	diazinon	fenuron	methomyl	phoxim	terbutryn
bitertanol	dichlorvos	flonicamid	methoxyfenozide	pirimicarb	thiabendazole
boscalid	dicrotophos	flucycloxuron	metolcarb	pirimiphos-methyl	thiacloprid
butylate	diethofencarb	flufenoxuron	metoxuron	prochloraz	thiamethoxam
carbaryl	difenoconazole	fluquinconazole	monocrotophos	profenofos	thiocyclam
carbendazim	dimethoate	fluroxypyr	monolinuron	propachlor	triadimenol
carbofuran	diuron	flutriafol	monuron	propamocarb	triazophos
chlorfenvinphos	dodemorph	imazalil	myclobutanil	propham	trichlorfon
chlorfluazuron	ethiofencarb	imidacloprid	nitenpyram	propoxur	trifloxystrobin
chlorotoluron	ethiofencarbsulfon	indoxacarb	omethoate	propyzamide	triflumizole
chloroxuron	ethiofencarbsulfoxide	iprodione	oxadixyl	pyridaphenthion	triforine
diuron-D6 (IS)					

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Set up of project

- Cover matrices relevant with respect to intake and varying complexity (21)

- Samples collected by each lab, homogenisation according to lab procedure
- One sample preparation method (QuEChERS, CEN version no dSPE clean up)
- Blank + spiked extracts at 3 levels: 0.01 mg/kg, 0.05 mg/kg, 0.20 mg/kg
- LC-MS/MS analysis using routine conditions of the lab

	Extract			Column (mm)				T	Eluent (gradient)		
MS/MS	g/mL	V_{inj}	pg *	L	ID	stationary phase	µm	(°C)	water/	additives	mL/min
Waters Premier XE	0.2	5	10	100	2.1	BEH C18	1.7	60	MeOH	5 mM NH4Fa	0.45
AB Sciex 4000	1.0	2	20	100	2.1	BEH C18	1.7	40	MeOH	10 mM NH4Fa	0.40
AB Sciex 5500	0.125	5	6.25	100	3	Atlantis T3	3	35	MeOH	5 mM NH4Ac/0.1% FA	0.40
Agilent 6490	0.125	5	6.25	150	2.1	Zorbax Rap.Res. HD Eclipse+ C18	1.8	50	MeOH	5 mM NH4Ac	0.50
Agilent 6490	0.10	10	10	150	4.6	Zorbax Eclipse XDB C8	5	amb	ACN	0.1% FA	0.60

* pg on-column for a sample containing 0.01 mg/kg

- 2 transitions for each pesticide (same transitions for all labs)

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Set up of project

Sequence:

- Solvent standards at beginning and end
- Spiked extracts, solvent standards randomized but same for all labs (120 inj.)

solvent 0	spinach 50 ppb	broccoli blank	solvent 50	onion blank	apple 10 ppb
solvent 2.5	kiwi 50 ppb	solvent 200	spinach 200 ppb	leek 200 ppb	solvent 10
solvent 5	melon 10 ppb	white cabbage 50 ppb	apple 200 ppb	paprika 50 ppb	pear 10 ppb
solvent 10	solvent 200	potato 200 ppb	rinse (acetonitrile)	white cabbage blank	tomato blank
solvent 25	apple 50 ppb	green beans 50 ppb	cucumber blank	broccoli 200 ppb	broccoli 10 ppb
solvent 50	strawberry 10 ppb	pear 200 ppb	kiwi 10 ppb	tomato 50 ppb	strawberry blank
solvent 100	solvent 10	solvent 50	green beans 200 ppb	tomato 10 ppb	cucumber 50 ppb
solvent 200	potato 50 ppb	onion 10 ppb	carrot 10 ppb	lettuce 50 ppb	lettuce blank
grapes 50 ppb	solvent 200	lemon blank	orange 10 ppb	carrot blank	lemon 50 ppb
cauliflower 200 ppb	spinach 10 ppb	cucumber 10 ppb	cauliflower 50 ppb	solvent 50	leek 10 ppb
broccoli 50 ppb	carrot 200 ppb	potato blank	solvent 50	leek blank	cauliflower blank
kiwi 200 ppb	melon 50 ppb	melon blank	potato 10 ppb	apple blank	onion 50 ppb
solvent 10	cauliflower 10 ppb	grapes 200 ppb	melon 200 ppb	lemon 200 ppb	solvent 0
lemon 10 ppb	lettuce 10 ppb	lettuce 200 ppb	leek 50 ppb	solvent 200	solvent 2.5
geen beans 10 ppb	carrot 50 ppb	paprika 10 ppb	tomato 200 ppb	solvent 50	solvent 5
green beans blank	paprika 200 ppb	white cabbage 10 ppb	onion 200 ppb	pear 50 ppb	solvent 10
strawberry 50 ppb	solvent 200	strawberry 200 ppb	rinse (acetonitrile)	solvent 10	solvent 25
kiwi blank	cucumber 200 ppb	rinse (acetonitrile)	paprika blank	orange blank	solvent 50
orange 200 ppb	rinse (acetonitrile)	spinach blank	white cabbage 200 ppb	pear blank	solvent 100
grapes 10 ppb	grapes blank	solvent 10	rinse (acetonitrile)	orange 50 ppb	solvent 200

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Set up of project

Data evaluation done in each lab:

- Manual verification of peak assignment/integration
- 21 matrices x (blank +3 levels) x 120 pesticides x 2 transitions = 21,600 EICs
- Exclude integrated noise, include any peak in blank within ±0.25 min of expected RT
- Export to Excel, send to RIKILT

EURL European Union Reference Laboratory – Pesticides in Fruits and Vegetables

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RIKILT Wageningen UR

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Data analysis RIKILT:

- Total 100,800 RTs and areas
- At individual lab-basis: determine reference RT, RRT, ion ratio
  => average of all solvent standards after removal of artefacts (too low signals, saturation)
- Calculate deviations of RT, RRT, ion ratio for individual pesticides/samples/conc.
- Investigate relationships deviations vs reference (R)RT and ion ratio
- Compare experimental data from 5 labs/systems with current EU criteria

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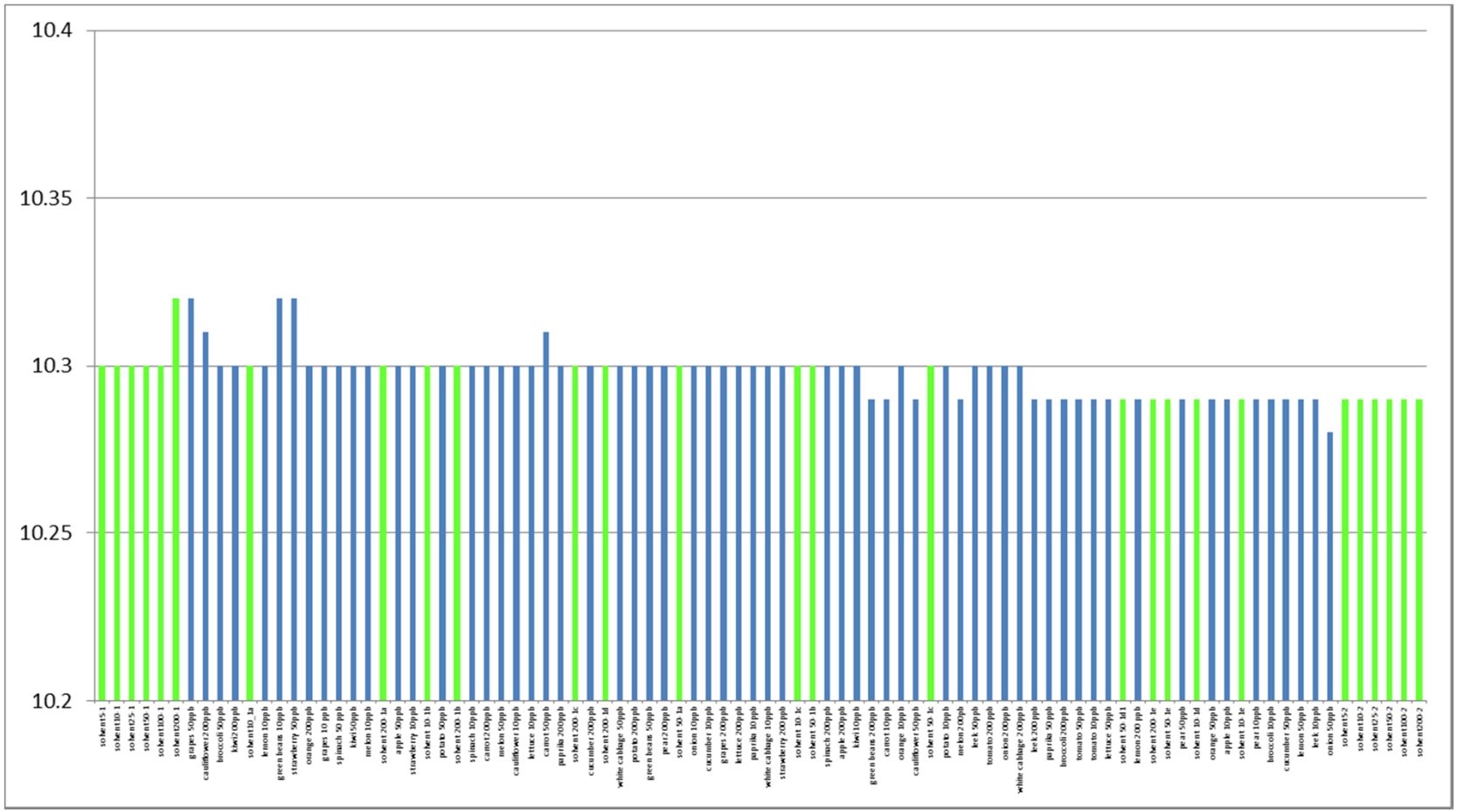
Example (relative) retention times observed

Pyriproxyphen: RRT solv.std ave = 1.813 RRT matrix ave = 1.812
RT solv.std ave = 10.297 min RT matrix ave = 10.298 min

1.858

10.56 +2.5%



Retention time in min →

↑ RRT ↓

10.04 - 2.5%

1.768

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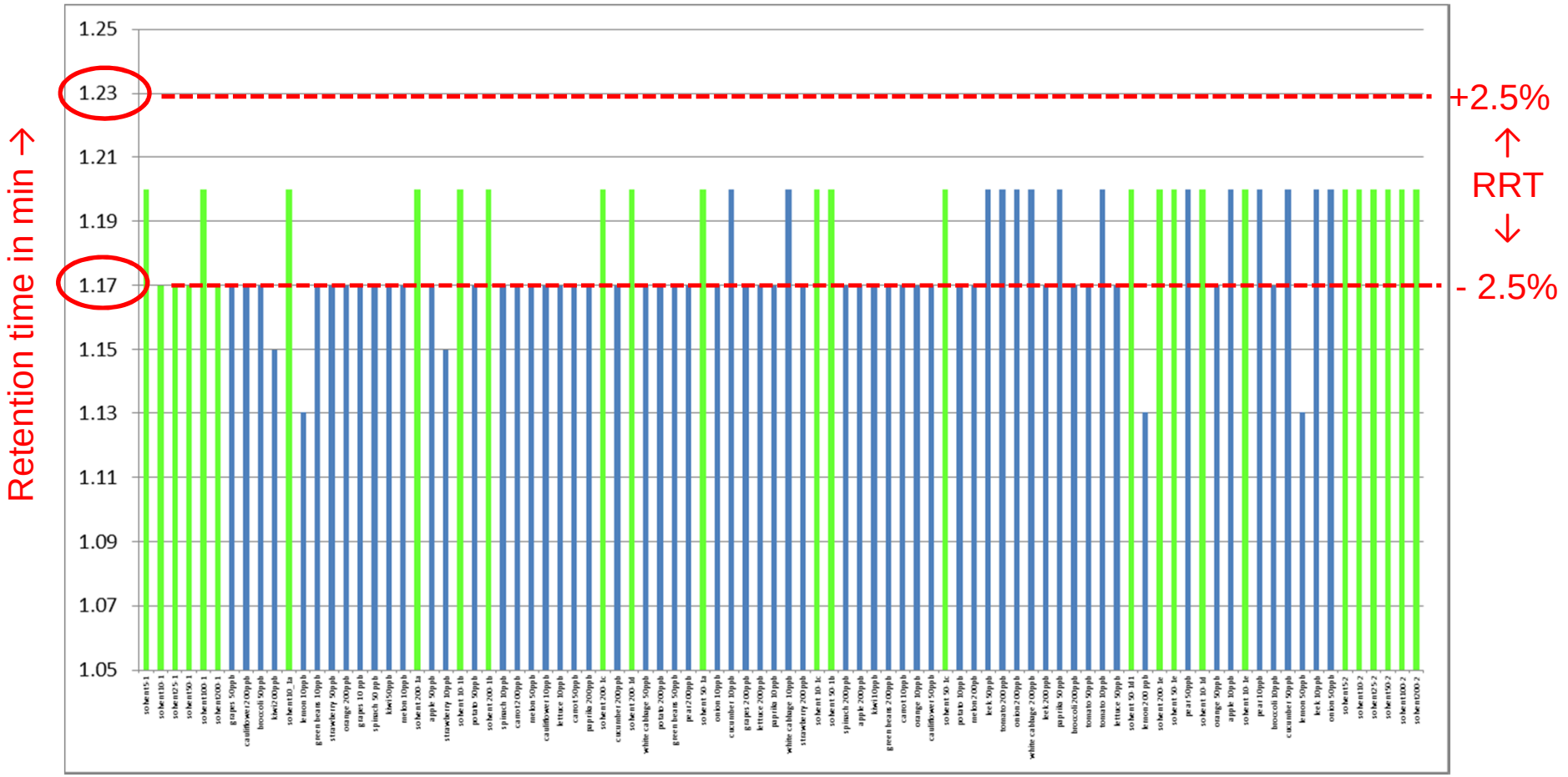
IS = D6-diuron , RT =5.68 min

Example (relative) retention times observed

Propamocarb: RRT solv.std ave = 0.211 RRT matrix ave = 0.207
RT solv.std ave = 1.174 min; RT matrix ave = 1.196 min



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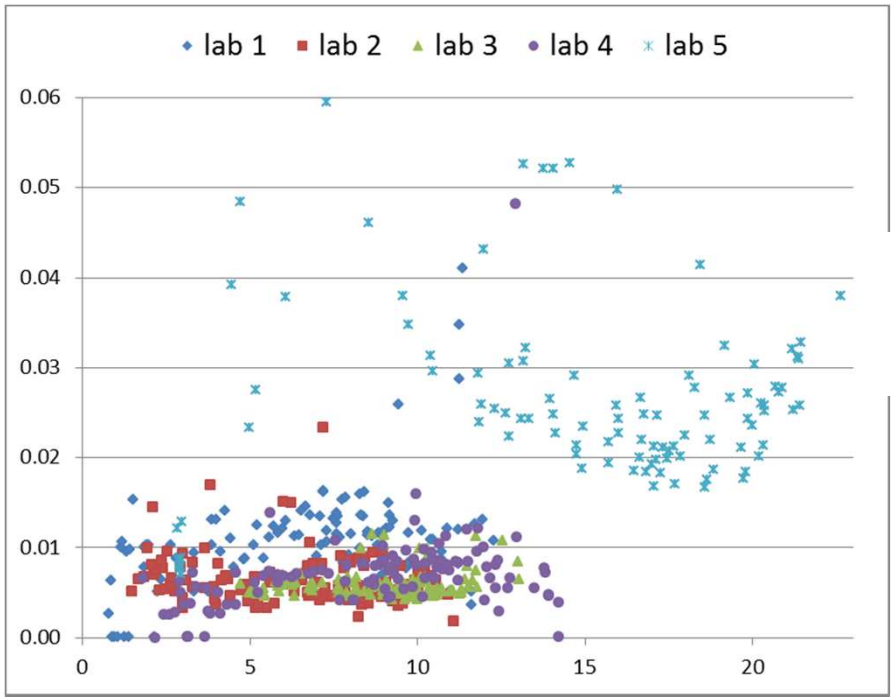
IS = D6-diuron , RT =5.68 min

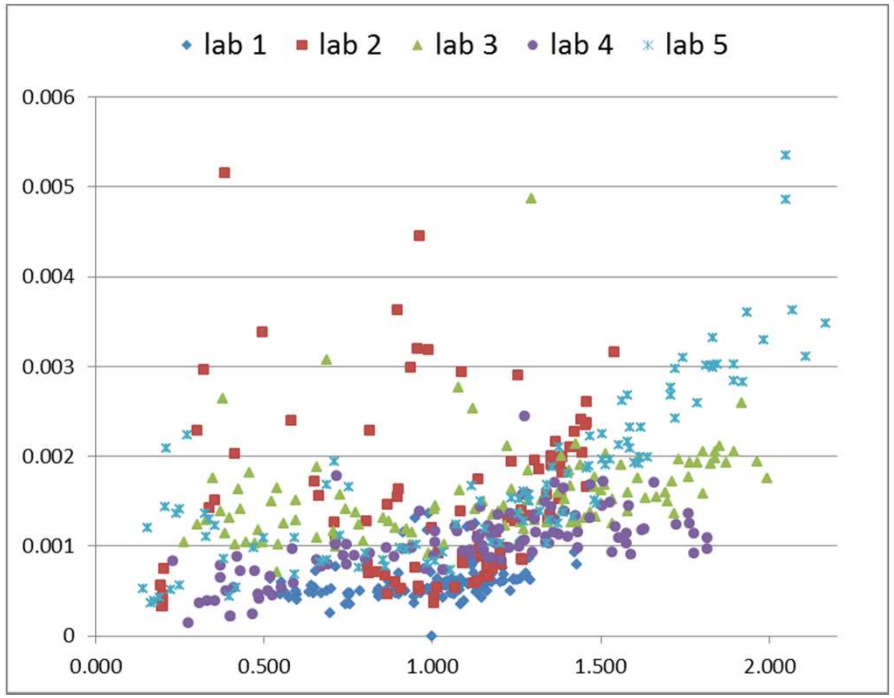
Stability of (relative) retention times

SD vs (relative) retention time for solvent standards
interspersed in a sequence of 120 injections of sample extracts



absolute retention time (min)

SD →



relative retention time (vs D6-diuron)

⇒ SD does not increase with RT (slightly with RRT?)

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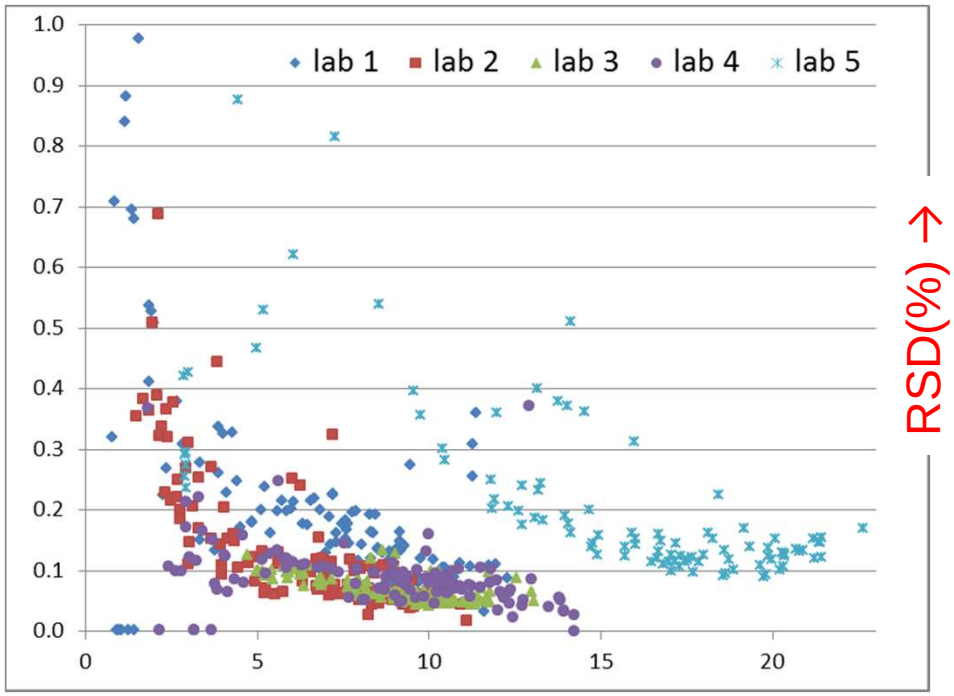
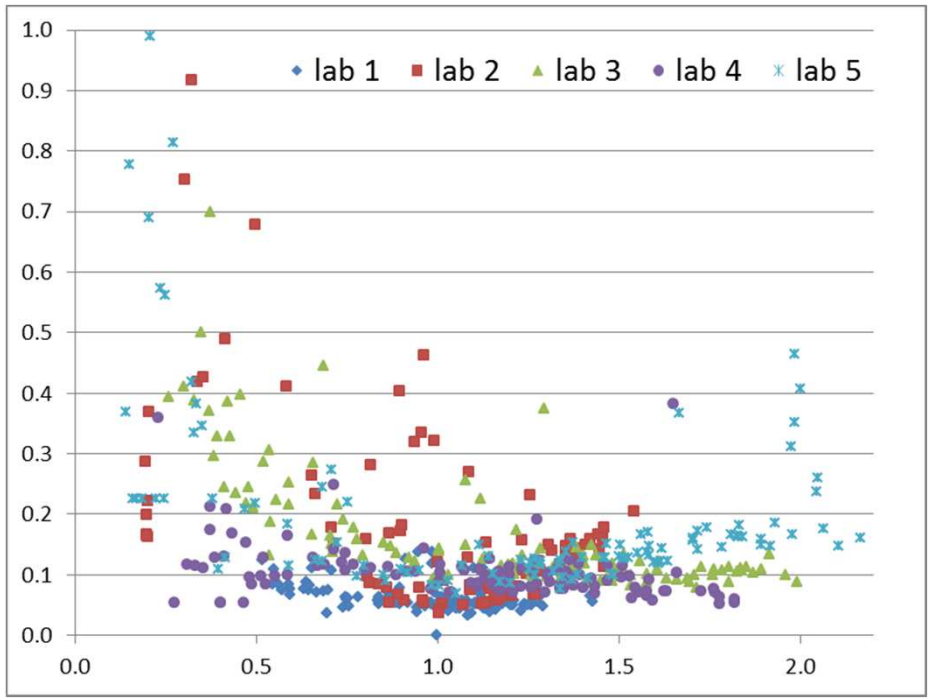
Stability of (relative) retention times

RSD vs (relative) retention time for solvent standards interspersed in 120 inj.-sequence



absolute retention time (min)

RSD(%) →



relative retention time (vs D6-diuron)

⇒ No increase of SD with RT => RSD descreases with RT

⇒ Slight increase of SD with RRT => RSD stable with RRT

⇒ No improvement of RSDs by using RRT (multi-method)

(R)RT criterion ±2.5% => 1.20±0.03 min .....10.30±0.26 min

⇒ Relative criterion for (R)RT does not make sense (gradient elution)

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Observed absolute retention time deviations

Retention time deviation of 120 individual pesticides in 21 different matrices at 3 concentration levels relative to their solvent-standard RT reference

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
RT deviation (min)*	percentage of all pest/matrix/conc. combinations				
0-0.025	99.97	99.75	99.13	99.21	87.94
0.025-0.05	0.03	0.25	0.13	0.44	8.48
0.05-0.1	0.00	0.00	0.75	0.33	2.69
0.1-0.15	0.00	0.00	0.00	0.02	0.34
0.15-0.20	0.00	0.00	0.00	0.00	0.09
0.2-0.25	0.00	0.00	0.00	0.00	0.21
>0.25	0.00	0.00	0.00	0.00	0.26

* deviation of pesticide in matrix from average RT of solvent standards

dominated by pirimicarb terbutryn, dicrotophos

0.9% for Lab 5

0.16% based on all lab results

⇒ Retention time deviations >0.10 min are exceptions in today's LC-MS analysis (gradient elution, using column oven)

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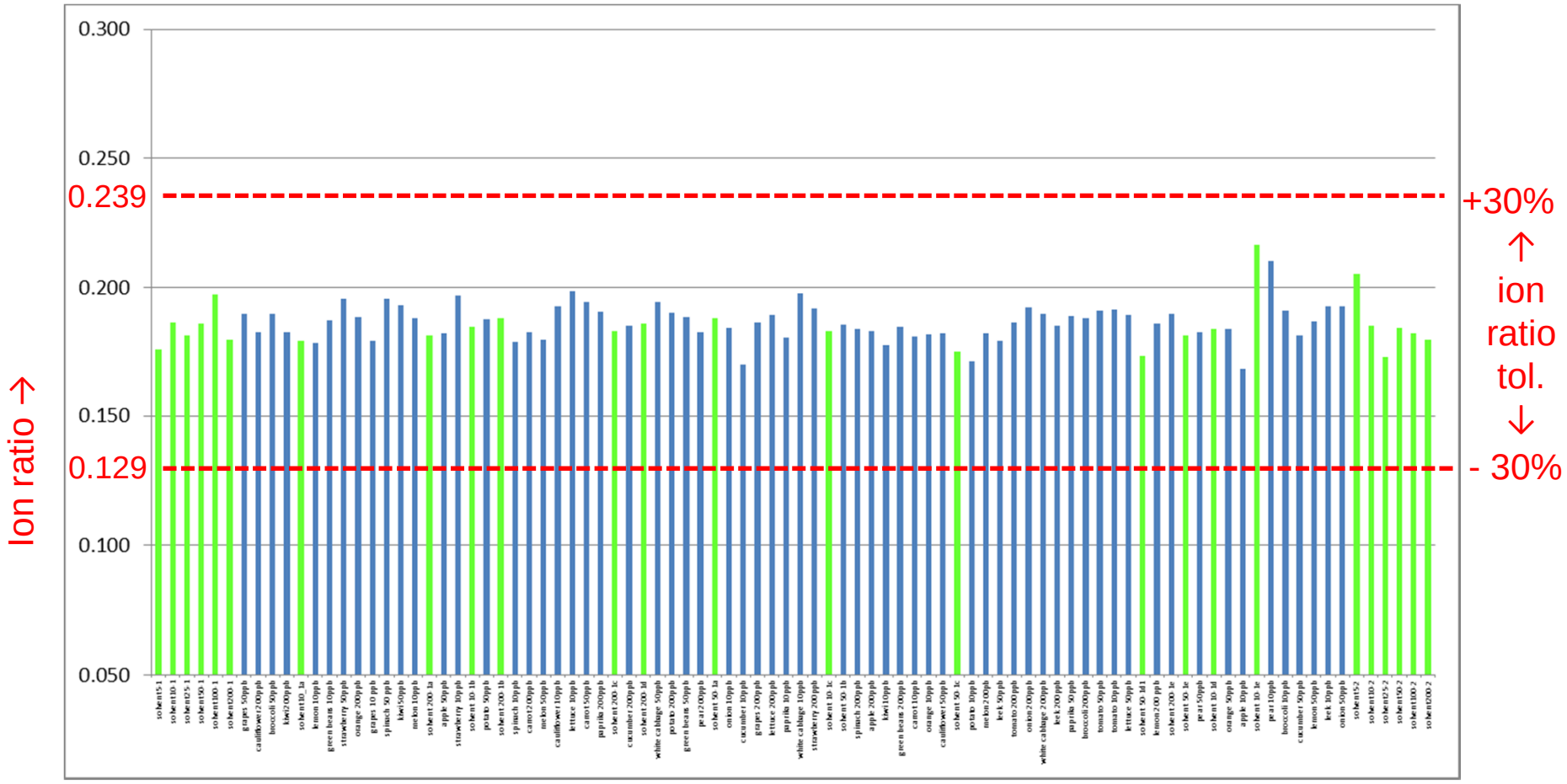
Example ion ratio variation observed

Pyriproxyfen: solv.std ave = 0.184 matrix ave = 0.187



⇒ No significant dependancy of ion ratio on concentration or matrix

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Partial snapshot of compilation made...

		General information on response and variability within the sequence									
		Transition-1				Transition-2					
		RF (area/pg) solvent standards				RF (area/pg) solvent standards				slope 2/slope 1	
analyte	Lab	average	RSD (%)	N	RRF	average	RSD (%)	N	RRF	ion-1	ion-2
pyriproxyfen	1	2625	21	26	5.677	483	21	26	1.046	0.89	0.91
pyriproxyfen	2	4529	13	27	1.793	20205	13	27	7.998	1.17	1.15
pyriproxyfen	3	216876	9	29	5.780	66519	6	29	1.773	0.92	0.93
pyriproxyfen	4	4529	10	29	4.301	275	13	28	0.261	0.84	0.69
pyriproxyfen	5	16662	16	29	16.007	4235	17	29	4.069	1.22	1.31

		Ion ratio									
		Solvent standards (=reference)								Samples	
analyte	Lab	average	RSD (%)	N	EU	EU min	EU max	min	max	min	max
pyriproxyfen	1	0.184	5	26	30	0.129	0.240	0.173	0.216	0.168	0.210
pyriproxyfen	2	0.224	4	27	25	0.168	0.281	0.205	0.237	0.207	0.242
pyriproxyfen	3	0.308	5	29	25	0.231	0.385	0.283	0.346	0.279	0.345
pyriproxyfen	4	0.060	12	28	50	0.030	0.091	0.050	0.083	0.048	0.074
pyriproxyfen	5	0.254	3	29	25	0.191	0.318	0.239	0.274	0.227	0.275

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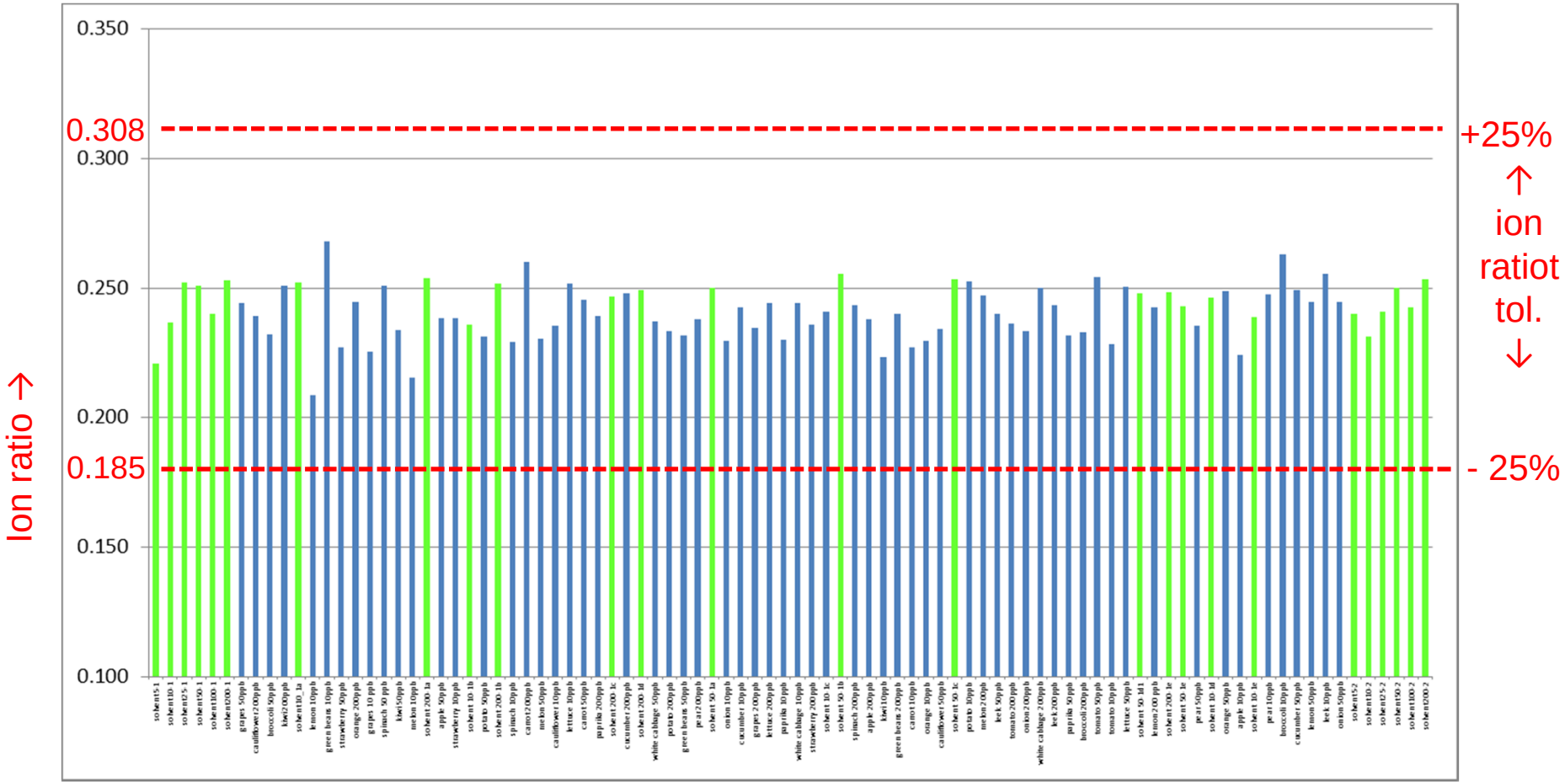
Example ion ratio variation observed

Propamocarb: solv.std ave = 0.246 matrix ave = 0.239



⇒ No significant dependancy of ion ratio on concentration or matrix

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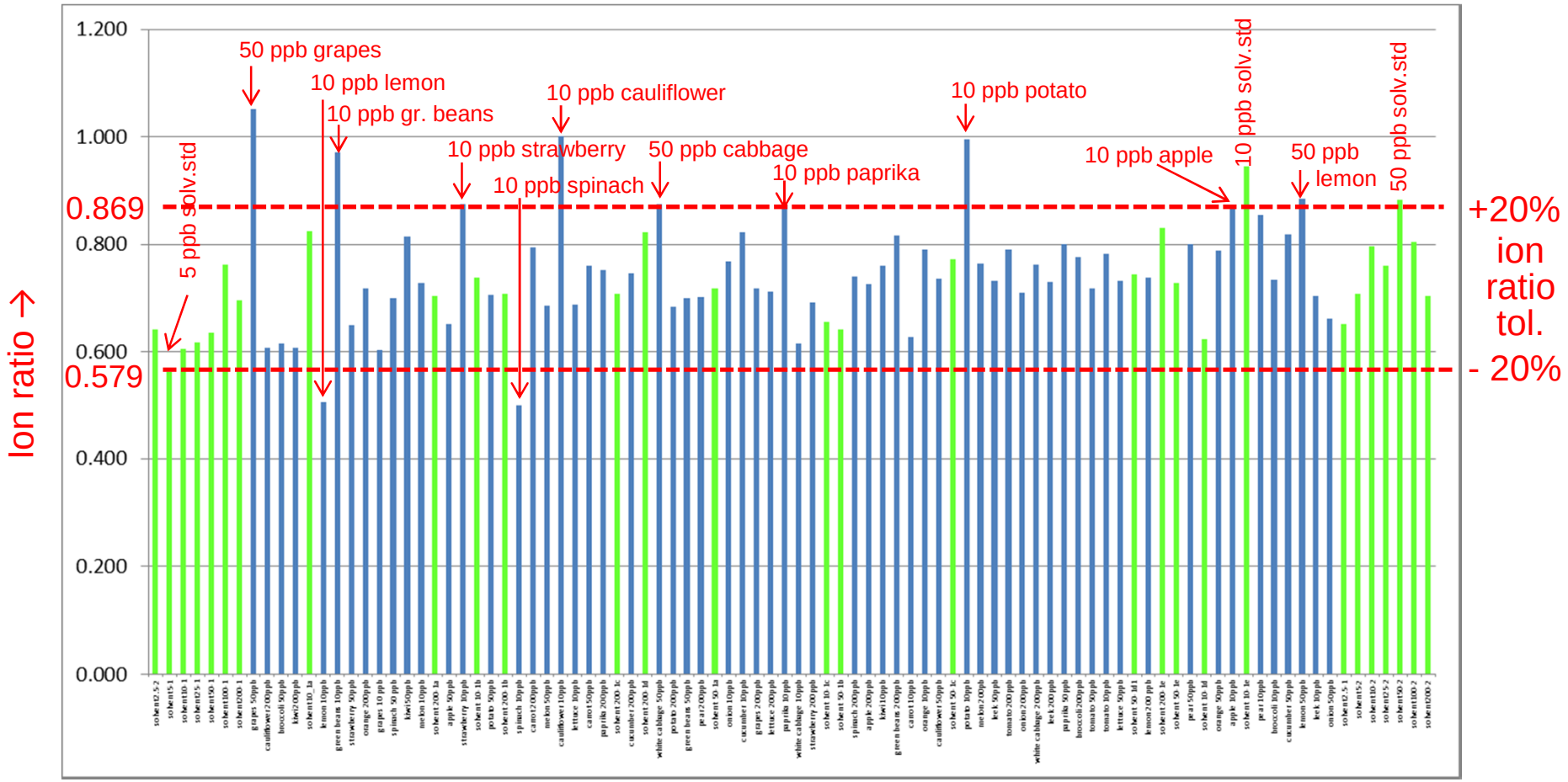
Example ion ratio variation observed

Kresoxim-methyl: solv.std ave = 0.724 matrix ave = 0.748



Reference: average solv.std 0.724, RSD=12% (N=29); **No signal in blank**

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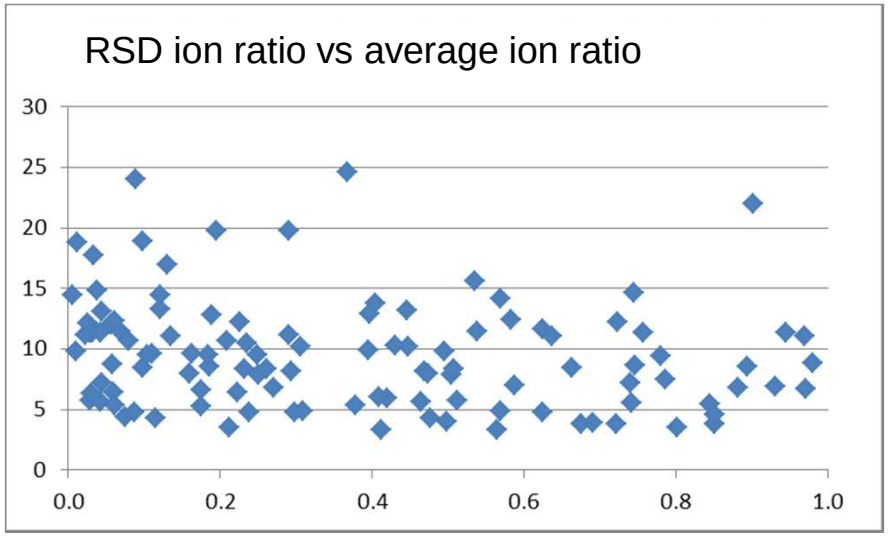
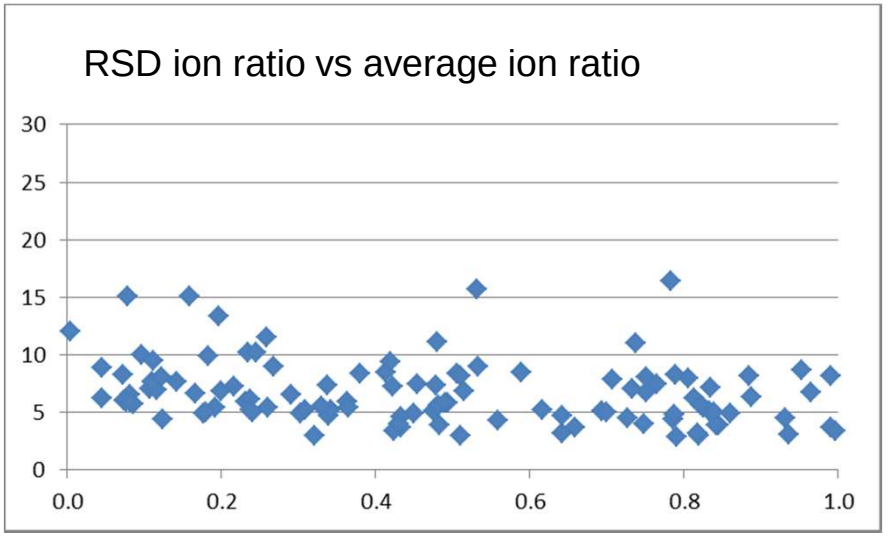
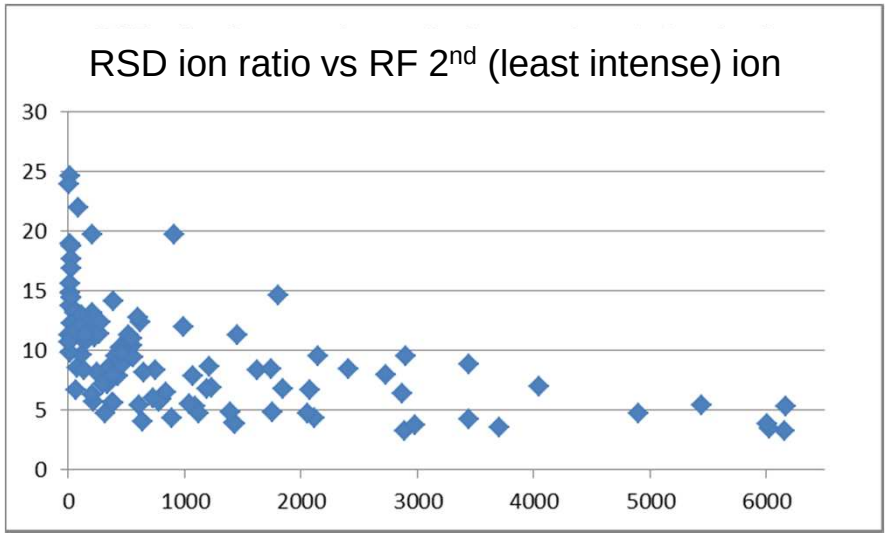
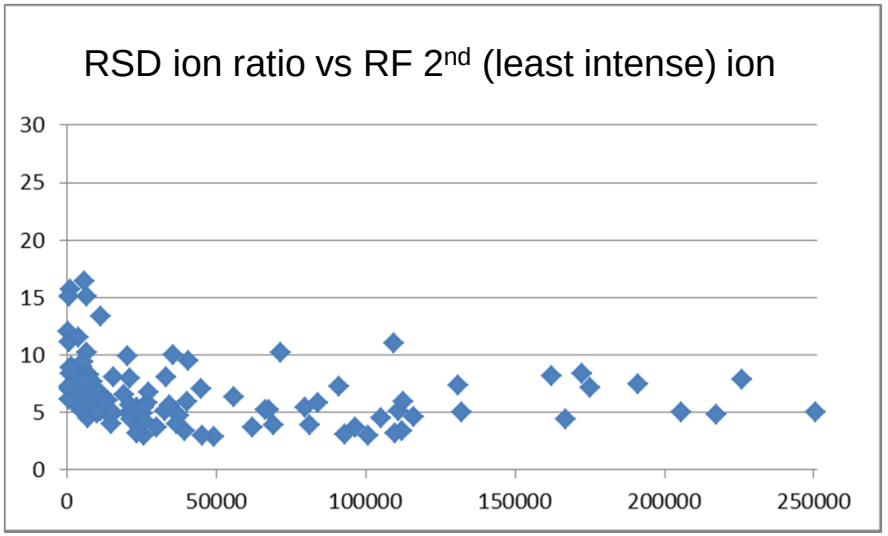
Outside EU criterion: 3x solv. std (5, 10, 50 ppb)
8x 10 ppb in matix; 3x 50 ppb in matrix

Assessment variation ion ratios (solv.stds)









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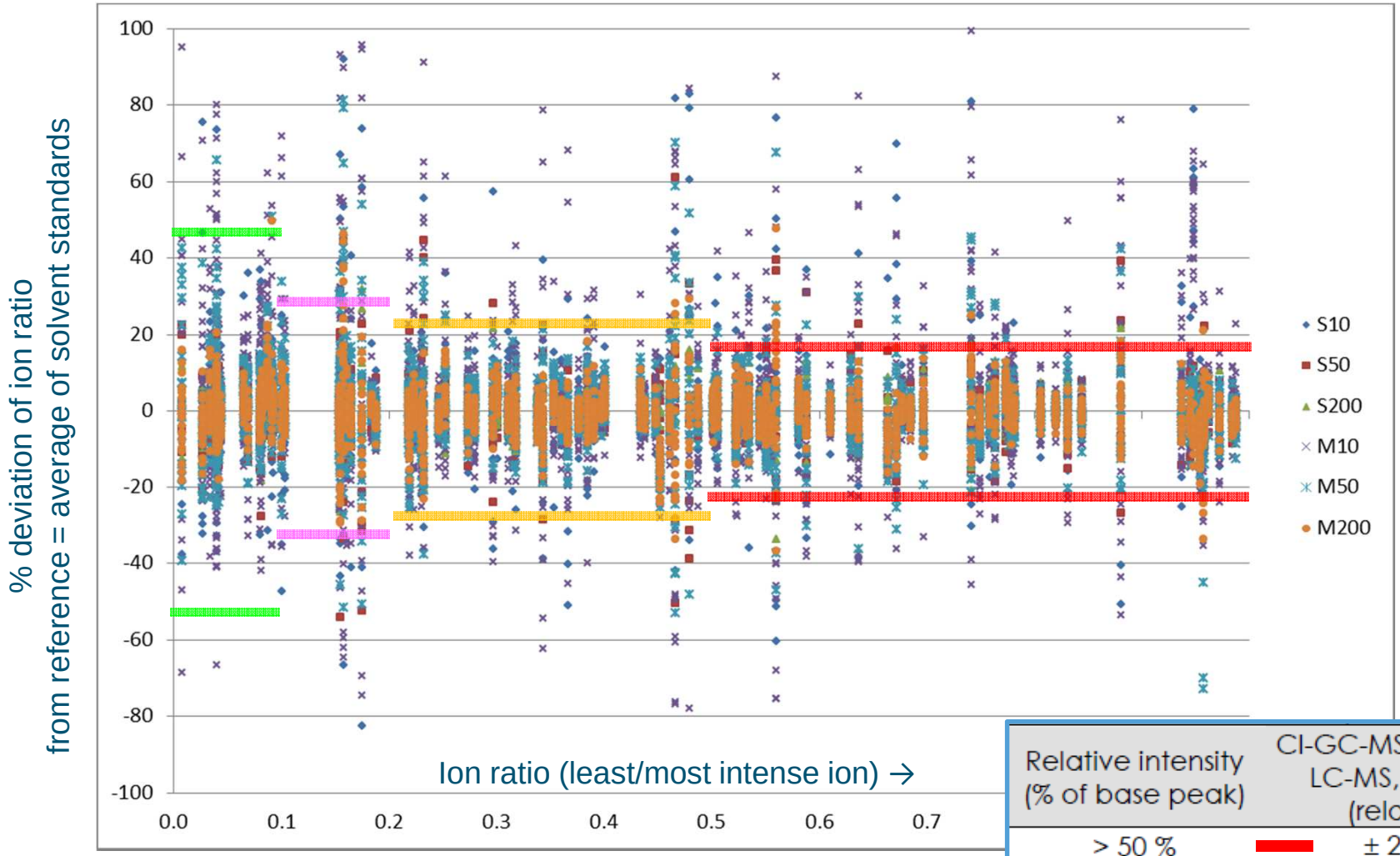
Agilent 6490

AB Sciex 5500 Qtrap

Investigation ion-ratio deviation from reference value

Waters Premier XE



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

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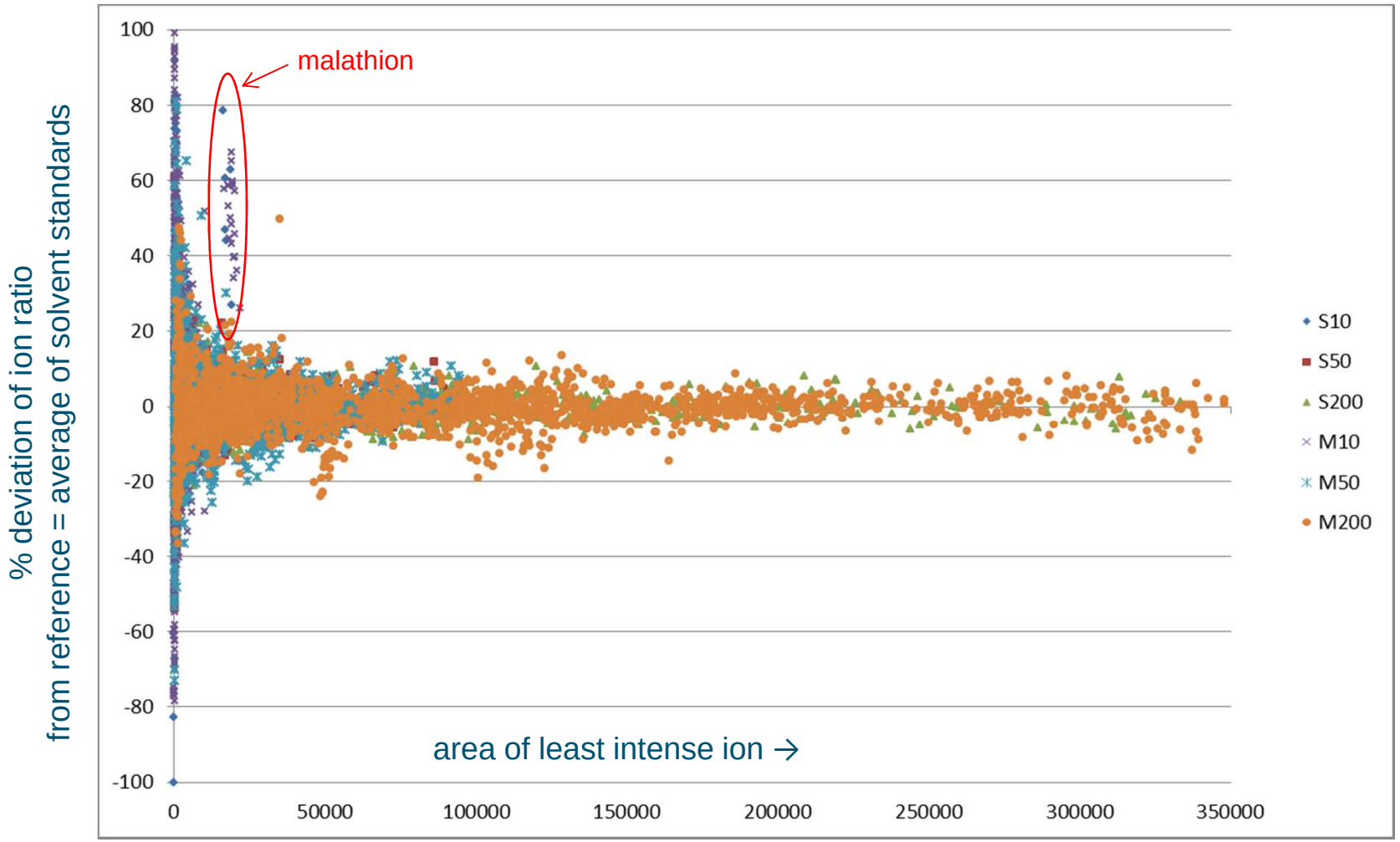
Ion ratio ≡ relative intensity

Investigation ion-ratio deviation from reference value

Waters Premier XE



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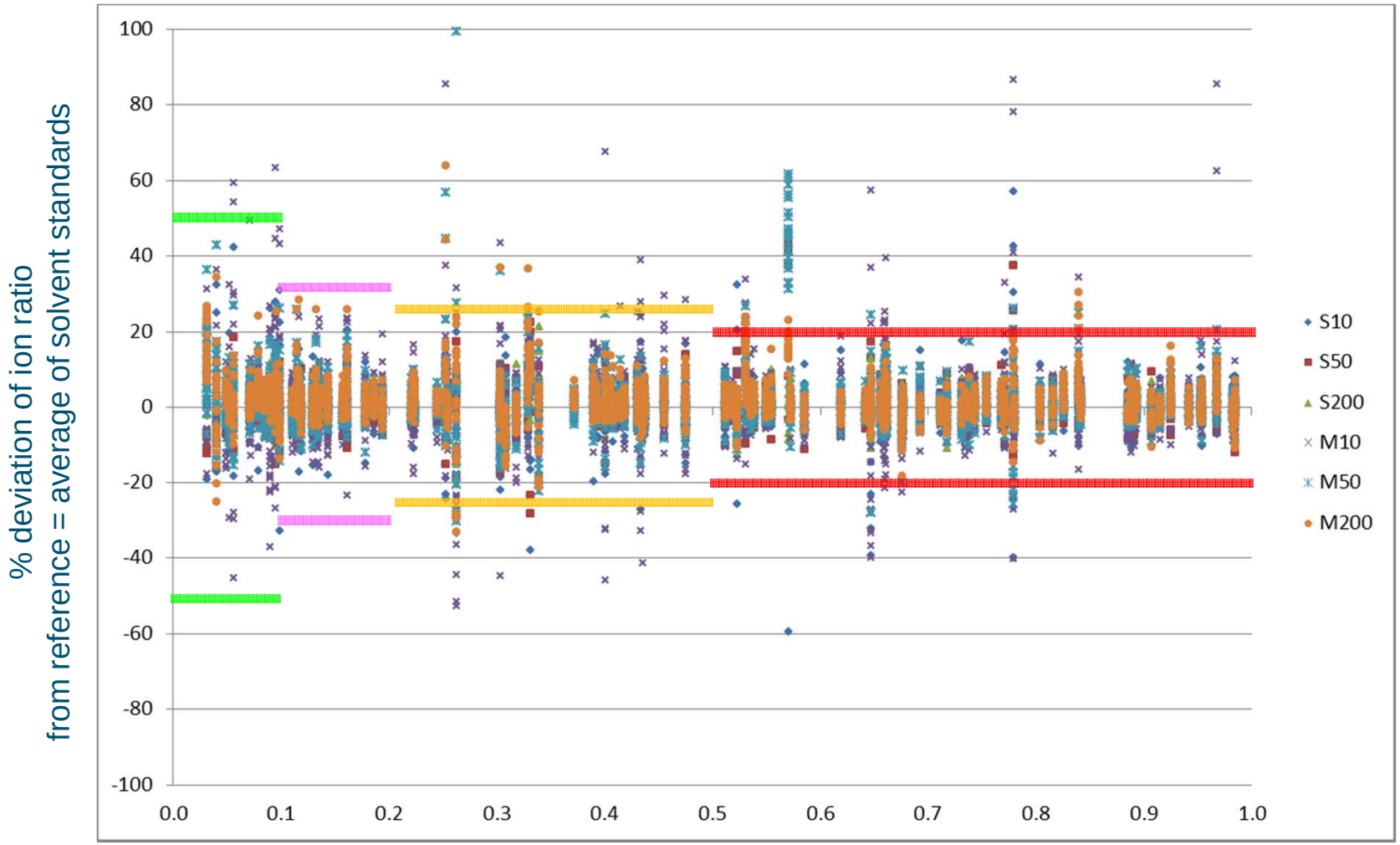
Investigation ion-ratio deviation from reference value

AB Sciex 4000



RIKILT
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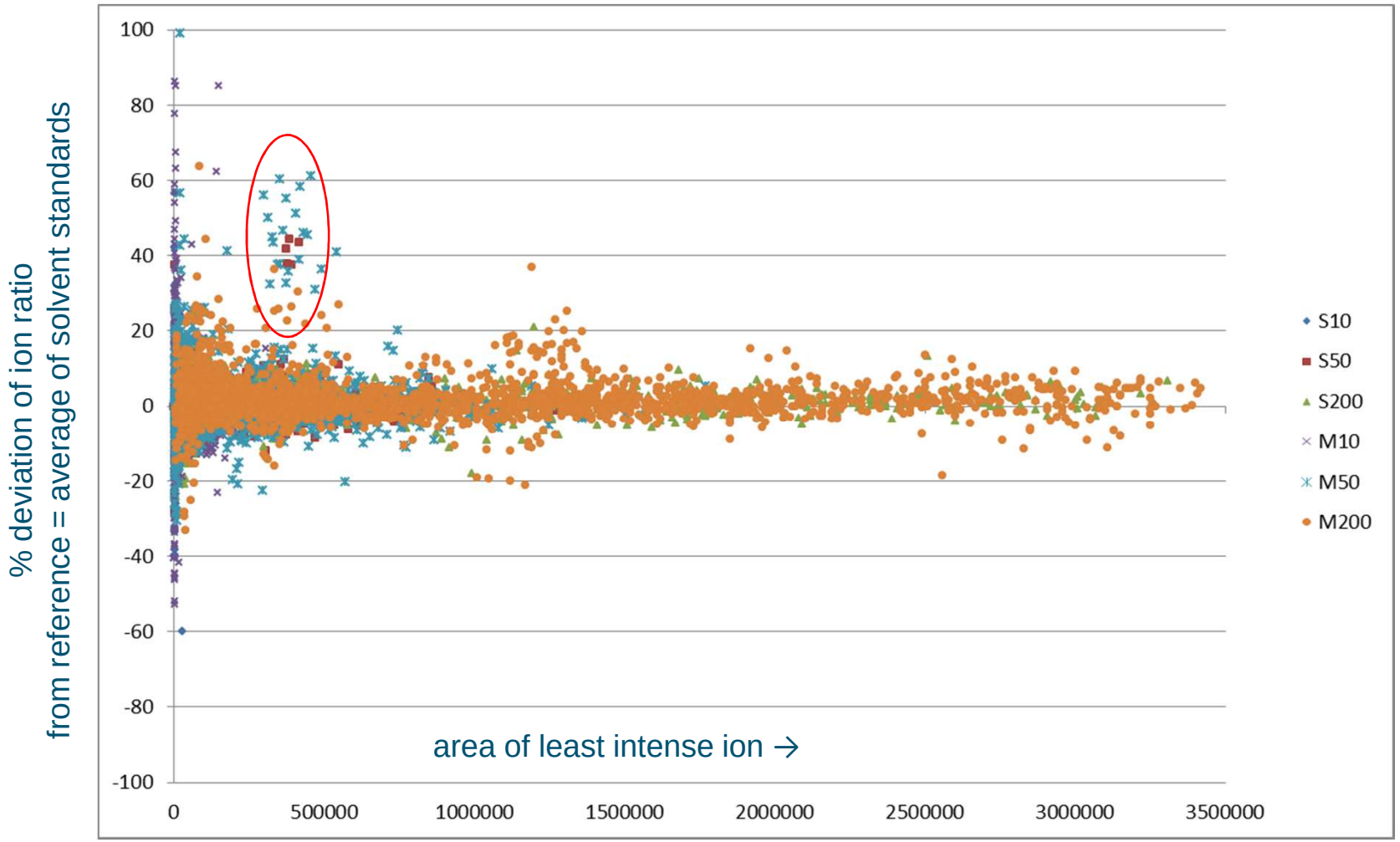
Investigation ion-ratio deviation from reference value

AB Sciex 4000



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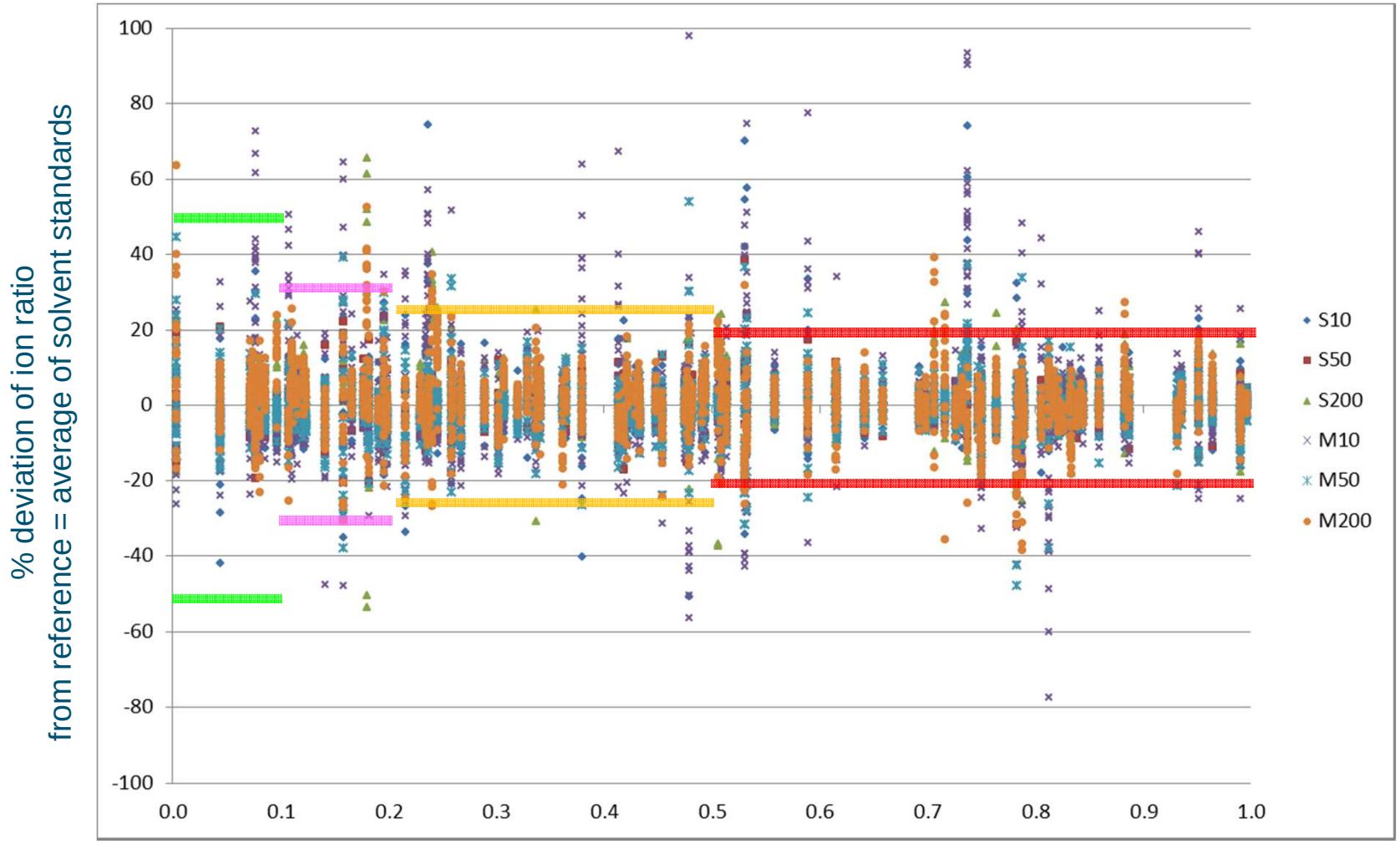
Investigation ion-ratio deviation from reference value

AB Sciex 5500 Qtrap



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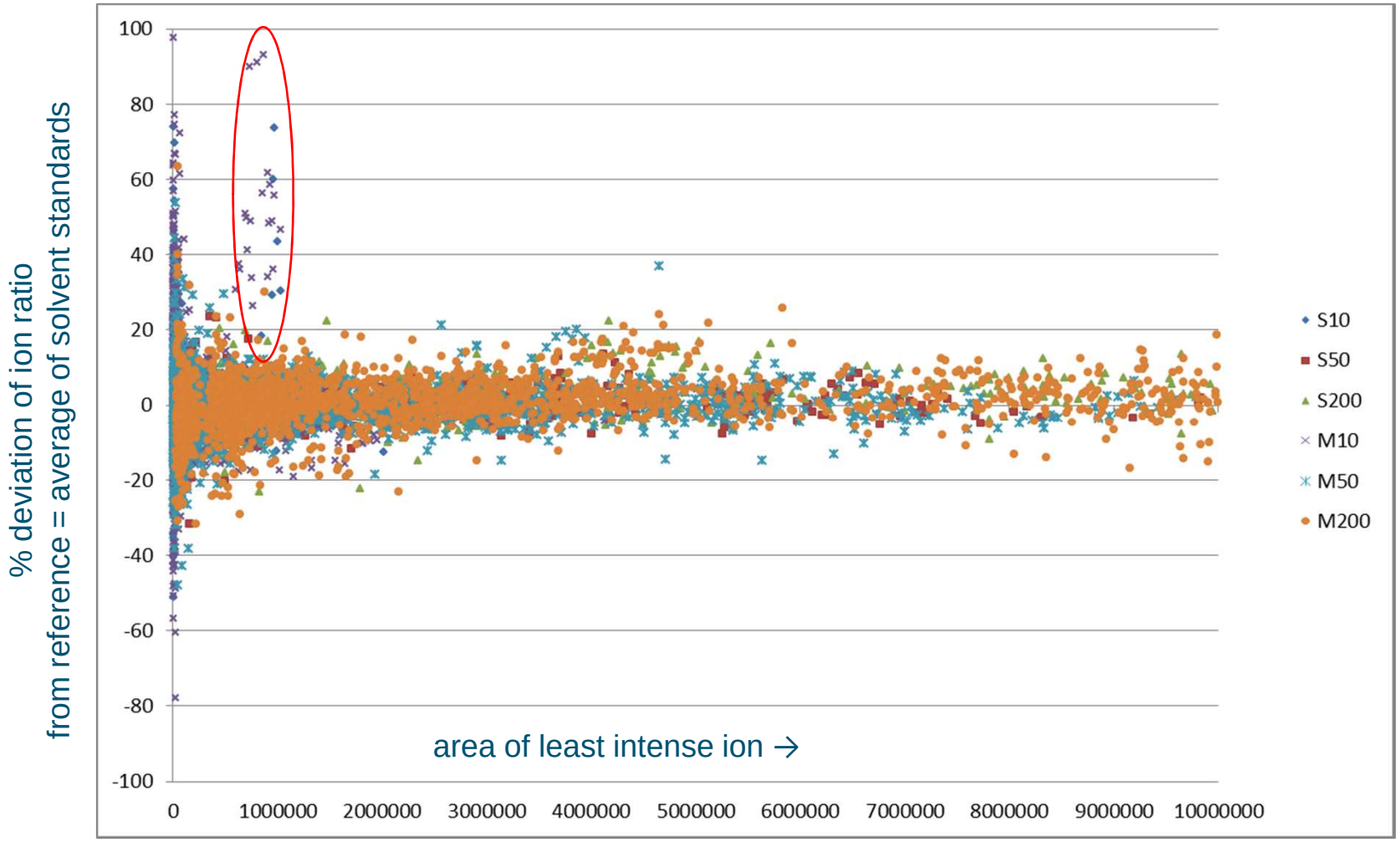
Investigation ion-ratio deviation from reference value

AB Sciex 5500 Qtrap



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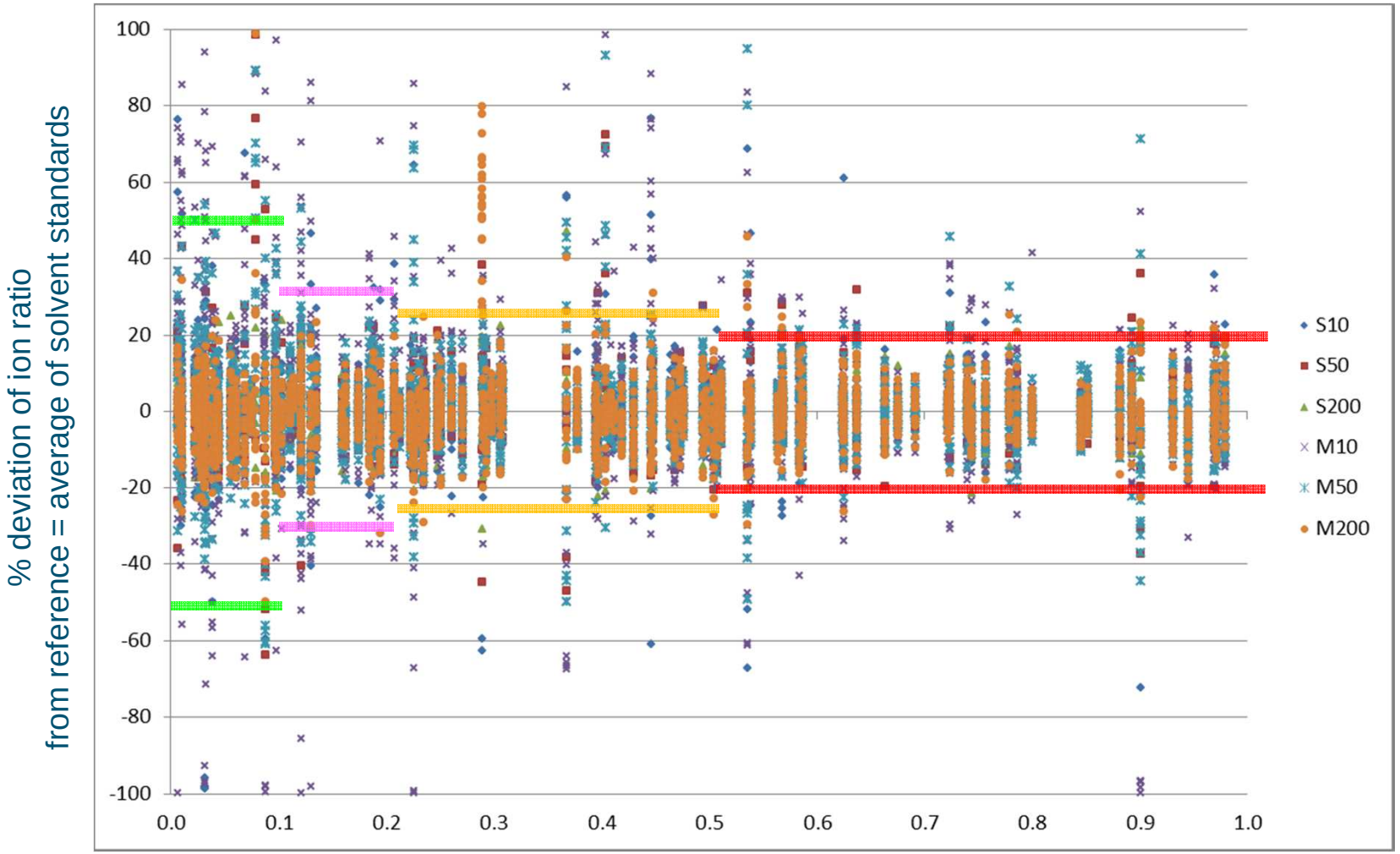
Investigation ion-ratio deviation from reference value

Agilent 6490 (lab A)



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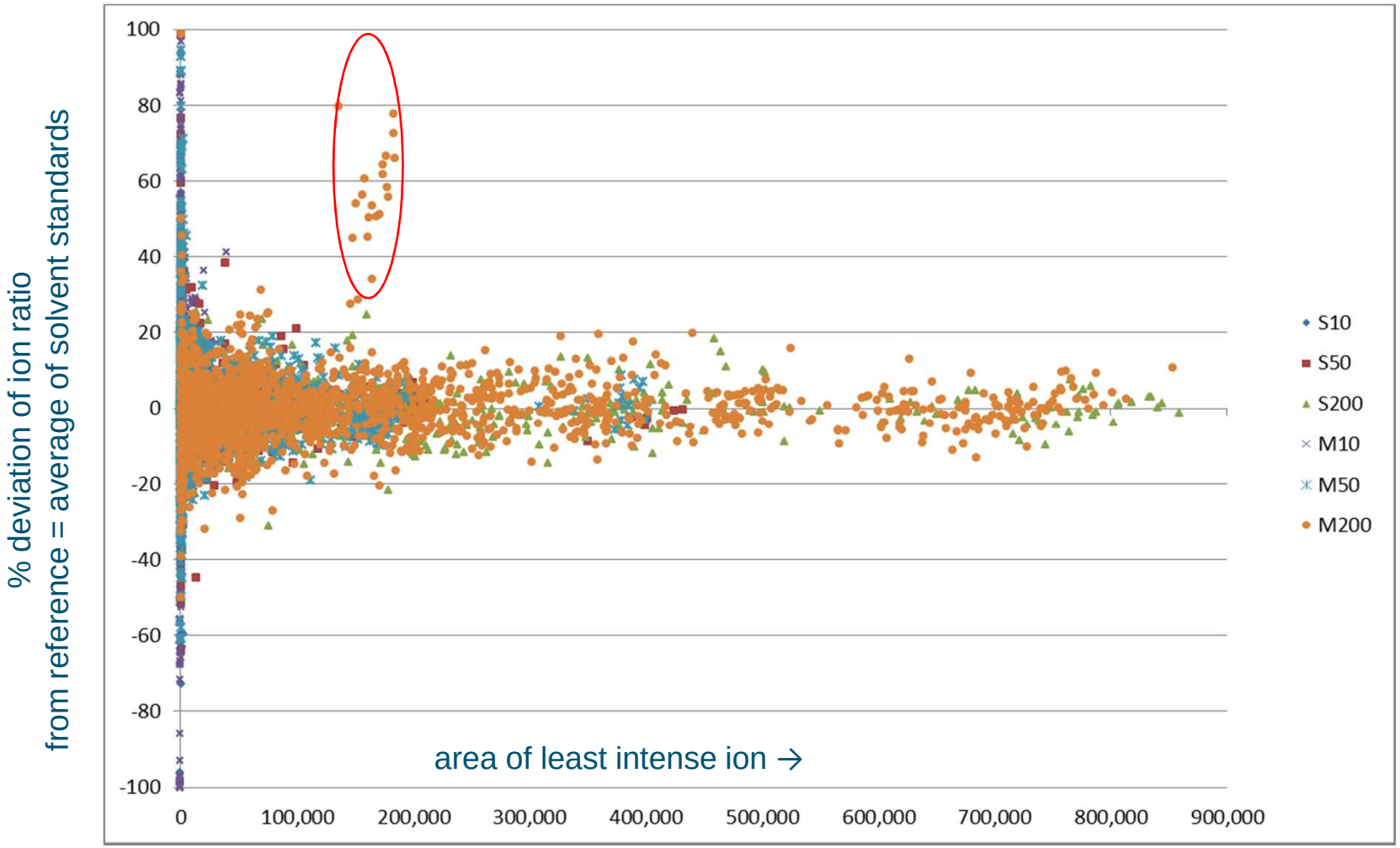
Investigation ion-ratio deviation from reference value

Agilent 6490 (lab A)



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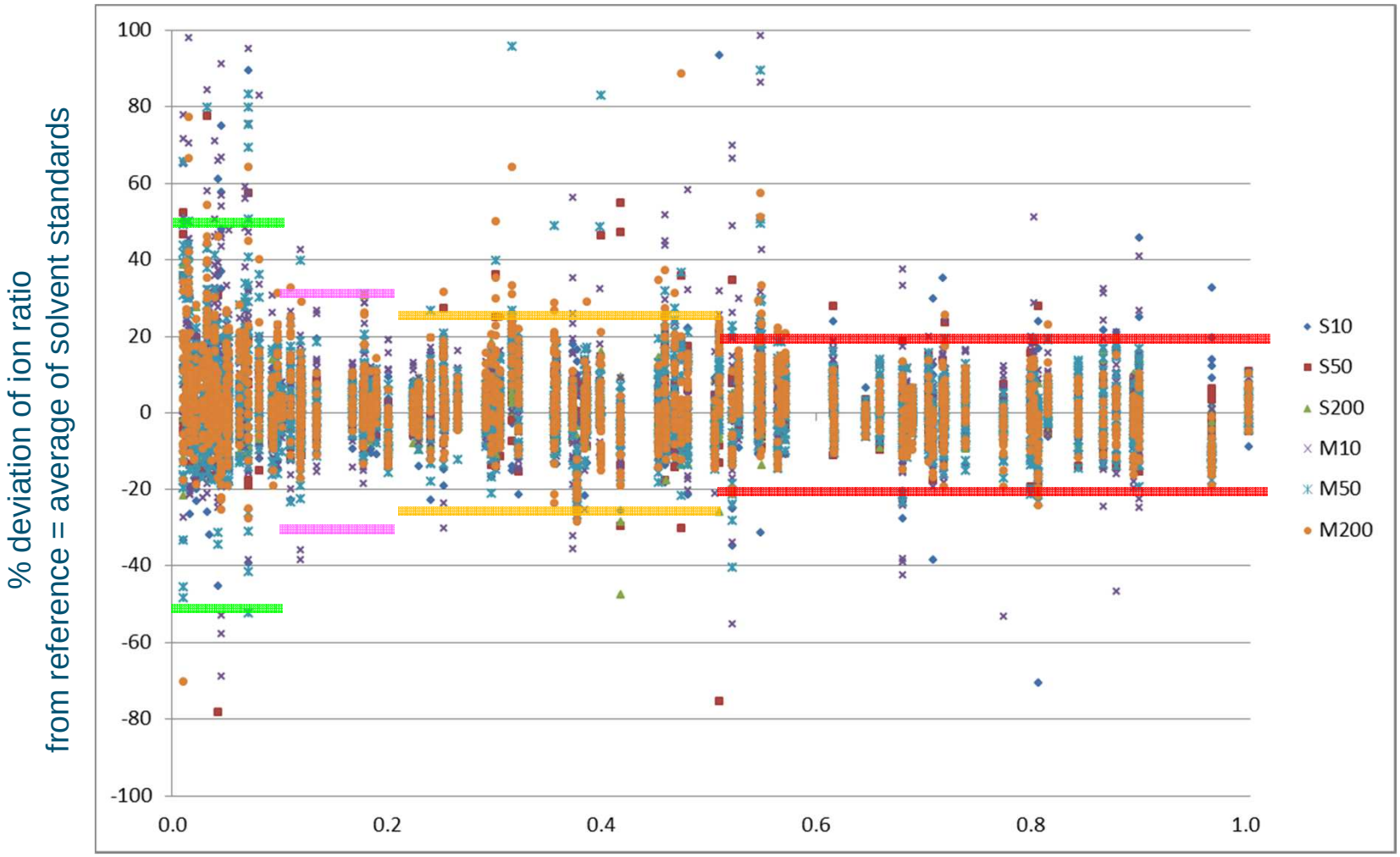
Investigation ion-ratio deviation from reference value

Agilent 6490 (lab B)



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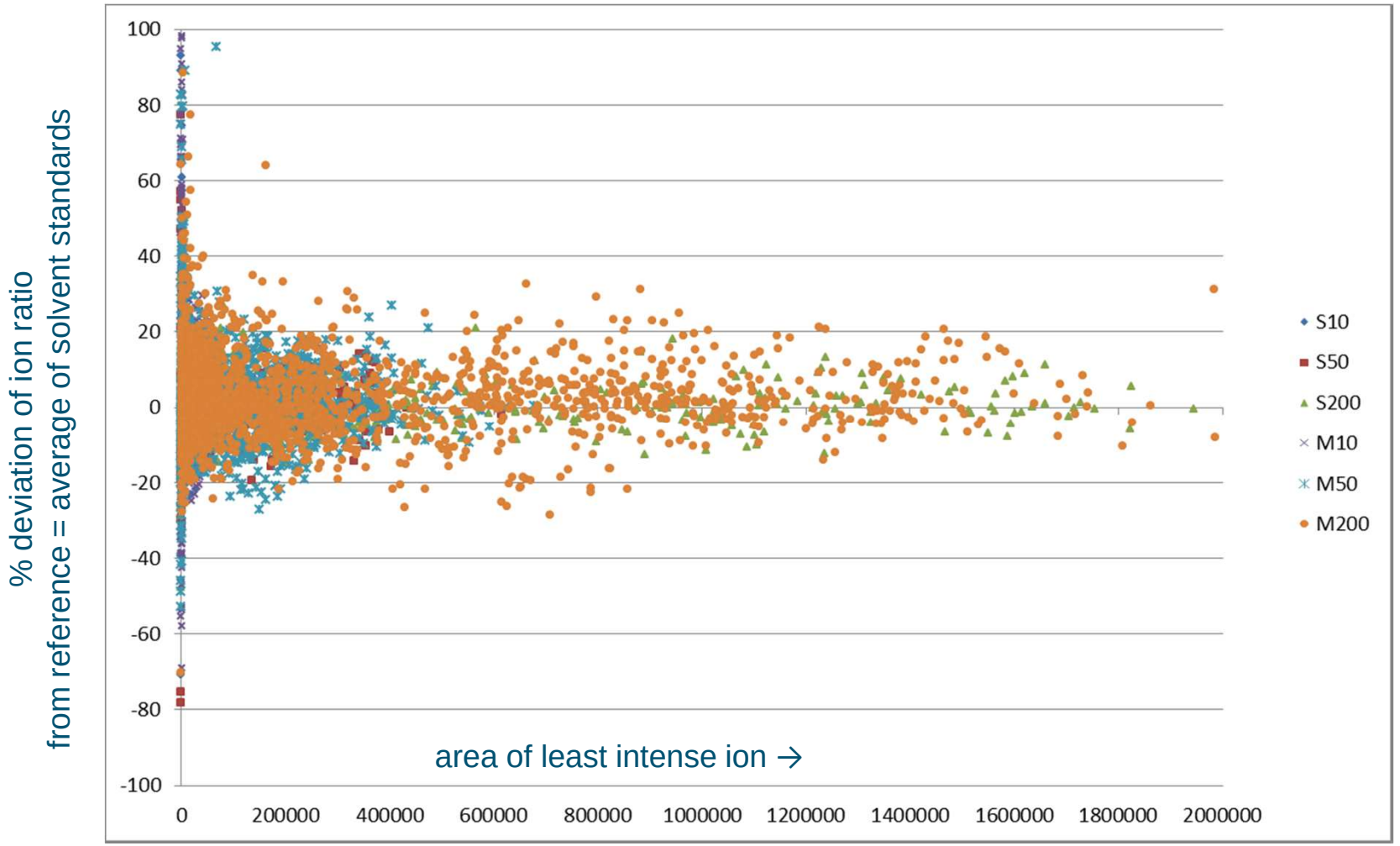
Investigation ion-ratio deviation from reference value

Agilent 6490 (lab B)



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Ion ratio tolerance vs identification

% of pesticides not identified as function of ion ratio tolerance for different response (area) ranges

	area range of least intense ion						
from	10	400	2000	6000	18000	54000	162000
to	400	2000	6000	18000	54000	162000	
	# data points for the range indicated above						
	393	1059	1017	1504	1545	1107	1659
%dev	% of pesticides not identified						
<±15	52.9	23.8	13.9	10.6	8.2	8.6	8.4
<±20	41.7	14.9	7.6	4.2	2.9	3.9	3.5
<±25	34.4	10.0	5.1	2.2	1.2	1.4	1.1
<±30	28.2	8.1	4.0	1.5	0.6	0.5	0.4
<±50	14.8	3.6	1.3	0.4	0.3	0.2	0.1
<±60	12.2	2.5	1.0	0.2	0.3	0.2	0.1
<±70	10.2	2.4	1.0	0.1	0.3	0.2	0.0

‘S/N’ < 3

3<S/N<15

15<S/N<45

45<S/N<135

135<S/N<405

405<S/N<1215

S/N>1215

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Agilent 6490 (lab B)

False negatives vs ion ratio tolerance

Data of spiked extracts from all labs combined (44,000 results)

S/N range	3-15	15-45	45-135	135-405	405-1215	>1215
all 5 laboratories/instruments #pest/matrix/conc combinations	5884	6955	9253	9279	6829	5798
ion ratio criterion	% false negatives					
<±15%	26.0	12.5	5.7	3.5	3.0	5.6
<±20%	16.3	6.4	2.2	1.1	1.1	2.5
<±25%	10.9	3.6	1.0	0.4	0.3	1.2
<±30%	8.1	2.2	0.5	0.2	0.1	0.6
<±50%	2.9	0.3	0.1	0.0	0.0	0.1
<±60%	2.2	0.3	0.1	0.0	0.0	0.1
<±70%	1.6	0.2	0.1	0.0	0.0	0.0

Acceptable % false negatives?

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(False) positives vs ion ratio tolerance

All data blank extracts compiled (12,600 pesticide/matrix combinations)

RT criterion	# transitions	Ion ratio criterion	Detected ≥ 0.002 mg/kg	Detected ≥ 0.010 mg/kg
≤ ± 0.1 min	1	-	121	66
	2	None	61	13
		EU	40	12
		≤ ± 20% (rel)	36	10
		≤ ± 25% (rel)	39	11
		≤ ± 30% (rel)	44	12
		≤ ± 50% (rel)	46	12

True positives

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Conclusions multi-lab assessment identification LC-MS/MS

Retention time:

- Retention times are highly stable, mostly within 0.05 min of REF RT, and virtually always within <0.10 min
- Retention time deviations are not related to the retention time, so a relative criterion in % does not make sense
- There is no reason for not setting criteria for absolute RT instead of RRT

Ion ratio:

- No obvious relation between relative abundance of the two transitions and the observed ion-ratio deviations
- The ion ratio deviation of an analyte in a sample from its reference value is mainly determined by MS response (S/N)

⇒ **It makes sense to adjust the criteria in line with these conclusions**

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Extrapolation to other types of MS ?

Table 4. 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
Ion ratio(s):		according to Table 5	

Table 5. Default recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques[2].

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 %

Outlook LC-single stage HRMS

21 fruits/vegetables
QuEChERS (AOAC, no dSPE clean up)
Blank + spikes at 0.01, 0.05, 0.20 mg/kg

5 µl inj. into LC-full scan hrMS (Exactive Orbitrap)
Alternating scan events:
1. Without fragmentation
2. With 'all ion fragmentation' (HCD cell, 30 eV)

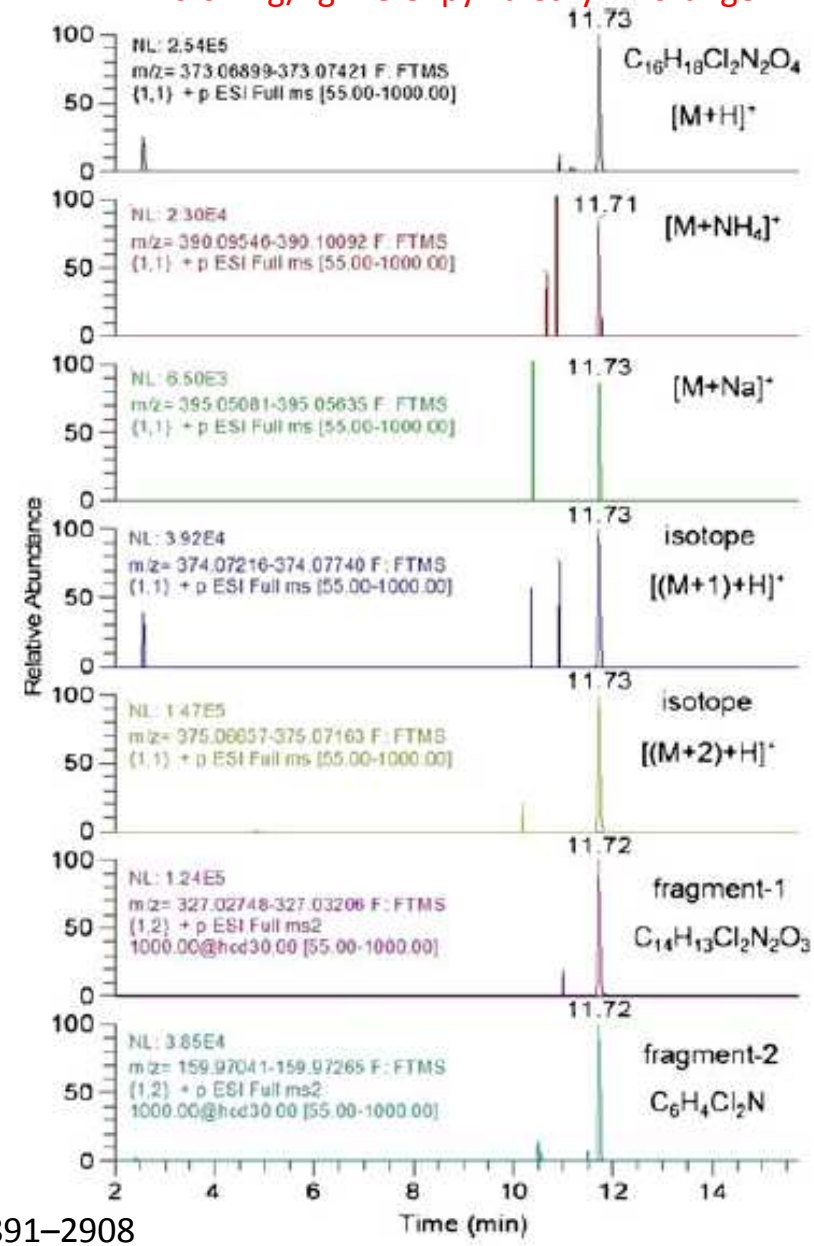
RP = 50,000 (m/z 200, FWHM)

- ➢ 2 diagnostic ions: multiple options....
- ➢ At least 1 fragment

⇒ Various options for ratio determination:

Frag-2 / Frag-1
Frag-1 / $[M+H]^+$
Frag-2 / $[M+H]^+$
.....

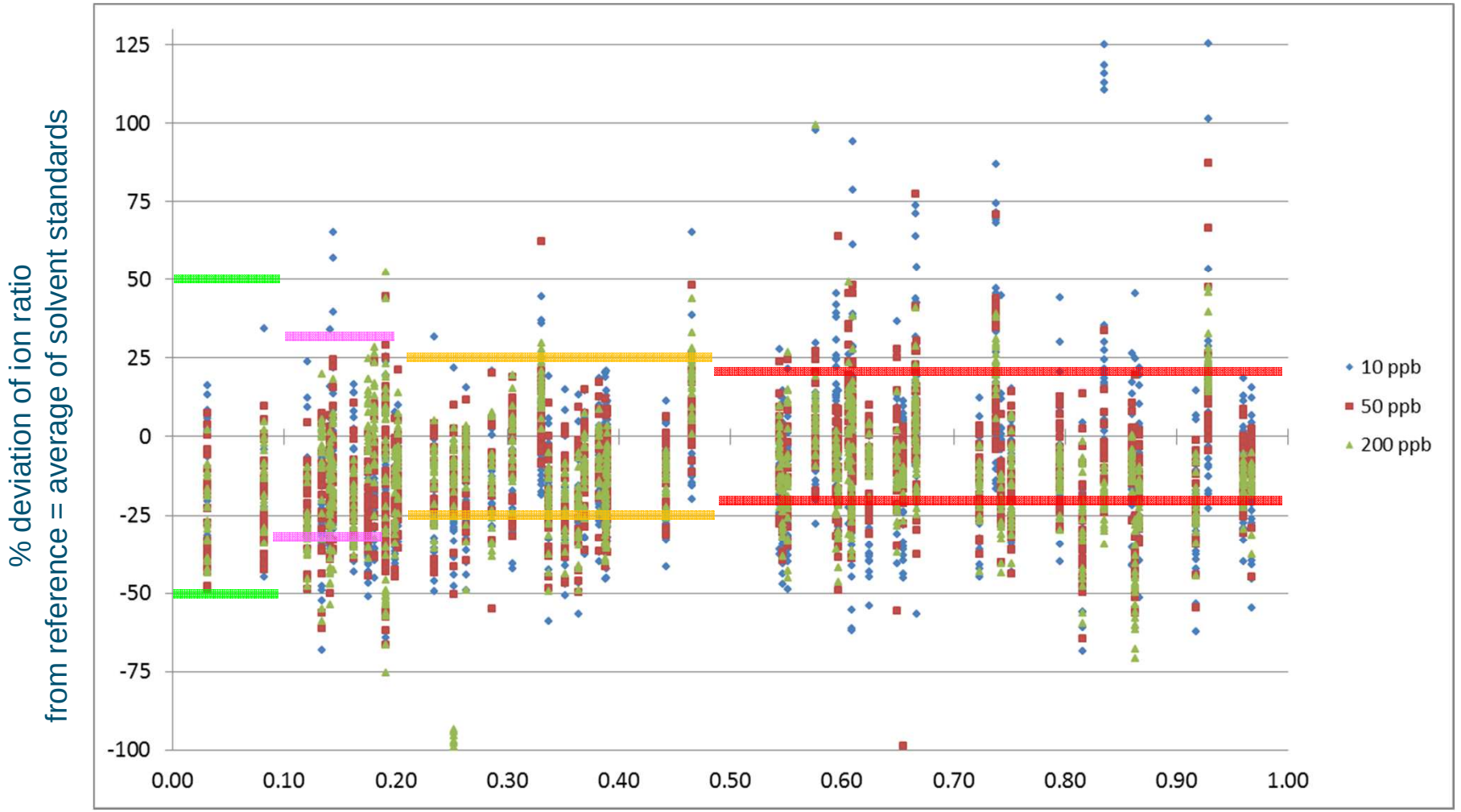
0.01mg/kg mefenpyr-diethyl in orange



Mol HGJ, Zomer P, de Koning M,
Anal Bioanal Chem (2012) 403:2891–2908

Investigation ion-ratio deviation from reference value

Thermo Exactive



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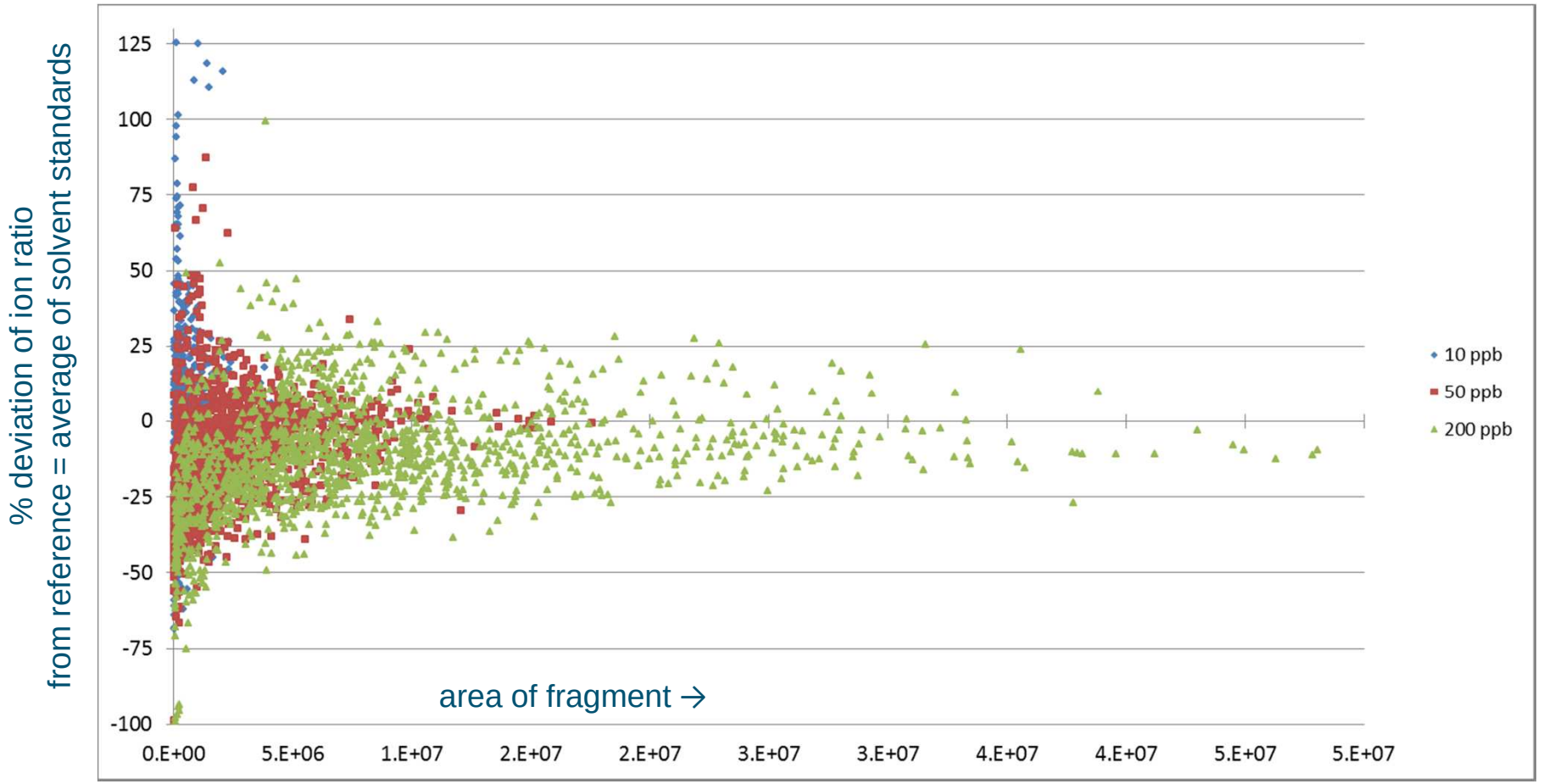
Investigation ion-ratio deviation from reference value

Thermo Exactive



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Ion ratio tolerance vs identification

LC-single stage HRMS
62 pesticides in 21 matrices, 3 levels diagnostic ions
Fragment-1 / $[M+H]^+$ (both <5 ppm mass accuracy)

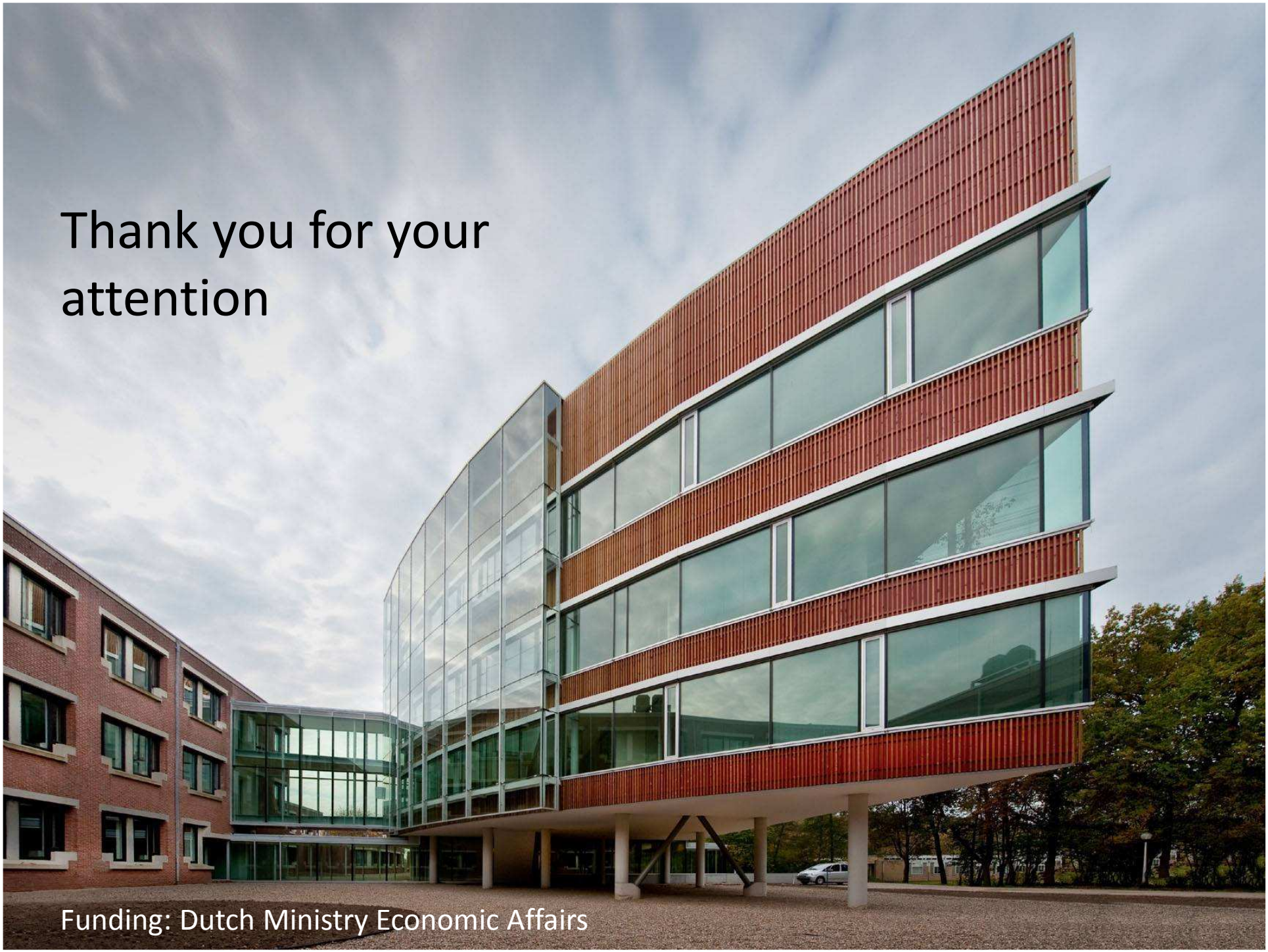
	no criterion		EU ion ratio criterion		fixed ion ratio tol. ±50%	
mg/kg	# detected	% detected	# detected	% detected	# detected	% detected
0.01	1145	88%	898	69%	1120	86%
0.05	1270	98%	1140	88%	1263	97%
0.20	1296	100%	1197	92%	1285	99%

Fixed ion ratio tolerance:
Investigated up to ±50% => no false positives
(for the 62x 21 = 1302 pest/matrix combinations investigated)

Justification/outlook GC-MS(/MS)

Multi-lab evaluation for GC-EI-MS/MS foreseen in 2014

Also assess GC-EI-MS (SIM) ??


Thank you for your attention
Funding: Dutch Ministry Economic Affairs

Proposed changes identification: Retention time

Default criterion for absolute retention time

Reference RT is average of solvent standards (or matrix-standard if free of interference)

Criterion: ≤ ± 0.10 min for both LC and GC

Remarks:
In LC use of column oven highly recommended
Be aware of analytes suseptable to RT shifts (pK_a vs pH, HILIC, ...)
Be aware of RT shifts due to heavy matrix loading
In case of greater RT deviations: IL-IS or overspikes may help you out

Proposed changes identification

Ion ratio deviations are mainly determined by S (S/N) not by the ratio of the two ions

In theory, a tolerance taking S/N into account would make sense
In practice not.....

⇒ One fixed tolerance: taking into account experimental data, experience, guidance in other areas: **±30% relative**

Extrapolation to other techniques (LC-HRMS(n), GC-MS/MS
But not (yet) to GC-EI-single stage MS (GC-MSD)

Table 5. Default recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques

Ion ratio (least/most intense ion)	Maximum tolerance (relative) for GC-EI-MS[1]	Maximum tolerance (relative) for LC-MS^n, LC-MS, GC-MS^n, GC-CI-MS
0.50-1.00	± 10 %	± 30 %
0.20-0.50	± 15 %	± 30 %
0.10-0.20	± 20 %	± 30 %
<0.1	± 50 %	± 30 %

Remarks on proposed changes identification

Remarks:
Reference = average of solvent standards measured in the same sequence
Matrix-standards only if demonstrated to be free of signal for diagnostic ions used

Impact of change of default ion ratio criterion (LC-MS/MS ion ratio project):

Fixed retention time criterion: RT± 0.1 min							
all levels		0.01 mg/kg		0.05 mg/kg		0.20 mg/kg	
# IDs out of 37800		# IDs out of 12600		# IDs out of 12600		# IDs out of 12600	
Ion ratio criterion							
EU	± 30%	EU	± 30%	EU	± 30%	EU	± 30%
33284	33484	10506	10600	11296	11332	11482	11552
88%	89%	83%	84%	90%	90%	91%	92%

Additional remarks

No rule will ever cover all pesticide/matrix/conc./instrument combinations,
there will always be exceptions to be decided on a case-to-case basis with proper justification, depending on what's at stake.

What if coinciding peaks for both transitions are present but ion ratio is incorrect?

- If concentration calculated for both transitions > MRL => must do additional ID exp.
- If concentration calculated for both transitions > RL =>
  should do additional ID experiments; or: increase RL, report as suspect or 'NA'
- If concentration for one transition < RL => report as < RL