Extraction of pesticides from cereals: combining maximum extraction efficiency with minimum degradation

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RIKILT results EUPT-cereals 2007-2008

	pesticide	incurred spiked	assigned µg/kg	Z-2*	Z+2*	method		rec%	recov correct?	reported µg/kg	Z-score
EUPT-C1 2007	Azoxystrobin	inc	240	120	360	ACN	LC	81	no	234	-0.1
	Carbendazim	inc/spi	126	63	189	ACN	LC	100	no	113	-0.4
	Deltamethrin	inc/spi	342	171	513	EtAc	**GC**	68	no	436	1.1
	Diazinon	inc	78	39	117	ACN	LC	63	no	87	0.5
	Pirimiphos-methyl	inc	6330	3165	9495	ACN	LC	65	no	7260	0.6
	Propiconazole	spi	353	177	530	ACN	LC	74	no	314	-0.4
	Endosulfan	inc	n.a.	n.a.	n.a.	EtAc	**GC**	96	no	35	n.a.
EUPT-C2 2008	Azoxystrobin	spi	239	120	359	EtAc	LC	82	no	234	-0.1
	Bifenthrin	inc	87	44	131	EtAc	**GC**	29	YES	94	0.3
	Carbendazim	inc	570	285	855	EtAc	LC	106	no	709	1.0
	Chlorpyrifos-methyl	inc	130	65	195	EtAc	**GC**	89	no	141	0.3
	Cypermethrin	inc	98	49	147	EtAc	**GC**	79	no	76	-0.9
	Difenoconazole	inc/spi	169	85	254	EtAc	LC	84	no	171	0.0
	Epoxiconazole	inc	176	88	264	EtAc	LC	95	no	187	0.3
	Iprodione	inc	289	145	434	EtAc	LC	75	no	355	0.9
	Malathion	inc/spi	162	81	243	EtAc	**GC**	62-68	no	< 50 (0.034)	-4.0
	Pirimicarb	inc	38	19	57	EtAc	LC	78	no	39	0.2
	Prochloraz	inc/spi	239	120	359	EtAc	LC	76	no	267	0.5
	Spiroxamine	inc	75	38	113	EtAc	LC	60	no	54	-1.1
	Trifloxystrobin	inc	439	220	659	EtAc	LC	77	no	432	-0.1

Routine method-1 (feedstuff)

1. Ethyl acetate

- Sample: 2.5 g
- Fortification: ISTD (REC-std) [wait 30 min]
- Wetting: 7.5 ml water, shake, soak until wetted
- Extraction: 20 ml ethyl acetate, shake 1 hr
- Phase separation: 10 g Na_2SO_4, vortex, centrifuge
- Clean up: concentrate: 16 ml -> 1 ml (TurboVap)
  GPC: EnviroGel, 450 x 19 mm ID; 5 ml/min
  collect fraction

Gas chromatography

30 ml -> 0.5 ml
Clean up: PSA 100 mg
vortex, centrifuge

10 µl GCxGC-TOF-MS (1 g/ml)

Liquid chromatography

15 ml -> just dry
Reconstitute: 0.1 ml acetone, vortex
0.4 ml methanol, vortex
0.5 H_2O/1% HAc, vortex

5 µl LC-MS/MS (0.25 g/ml)

Routine method-2 (feedstuff)

1. Acetonitrile (Quechers)

- Sample: 2.5 g
- Fortification: ISTD (REC-std) [wait 30 min]
- Wetting: 7.5 ml water, shake, soak until wetted
- Extraction: 10 ml acetonitrile/1% HAc, mix by hand
- Phase separation: 4 g $MgSO_4$, 1 g NaAc, vortex (3 min), centrifuge
-

Gas chromatography
0.5 ml

Lacks robustness for feedstuff, but might work with additional clean up*

10 µl GCxGC-TOF-MS (0.25 g/ml)

Liquid chromatography
0.5 ml filter into autosampler vial

5 µl LC-MS/MS (0.25 g/ml)

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*Stanisław Walorczyk, J. Chromatogr. 2008 (in press)

Follow up bad Z-score malathion

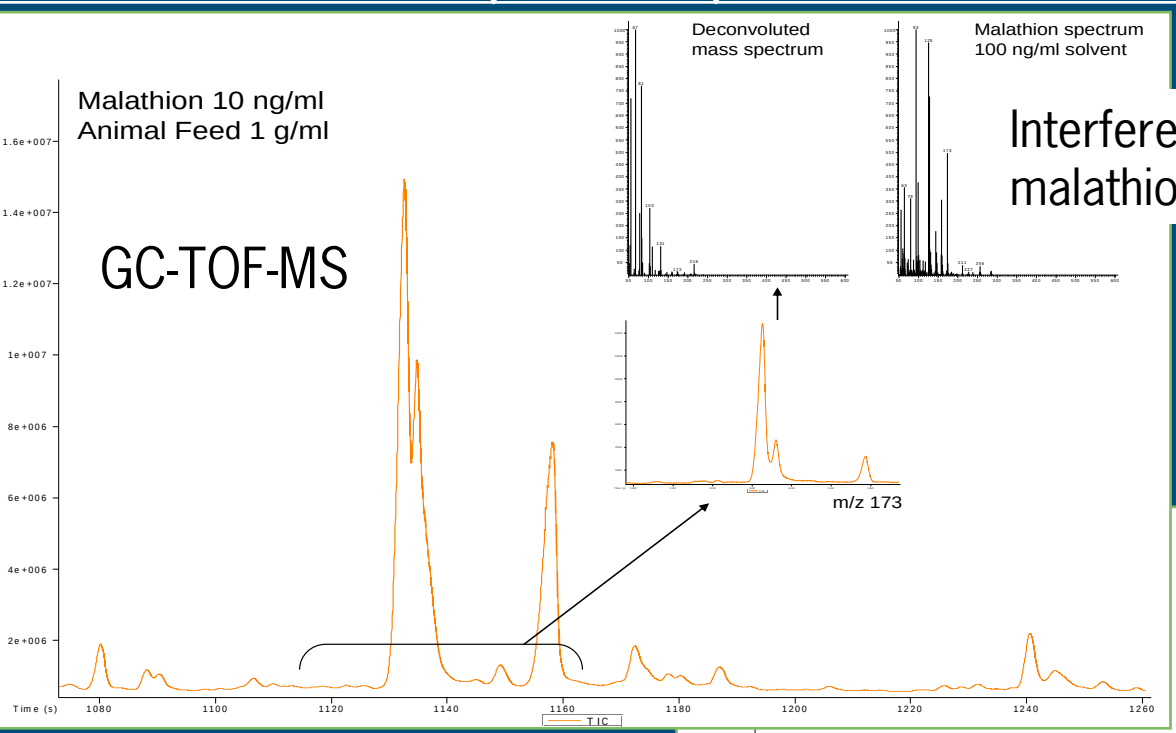
Possible causes:

- Trivial error (reporting, copy/paste)
- Measurement (interference, integration)
- Calculation (standard solution, linearity, matrix effects, response drift)
- Sample pretreatment (recovery)

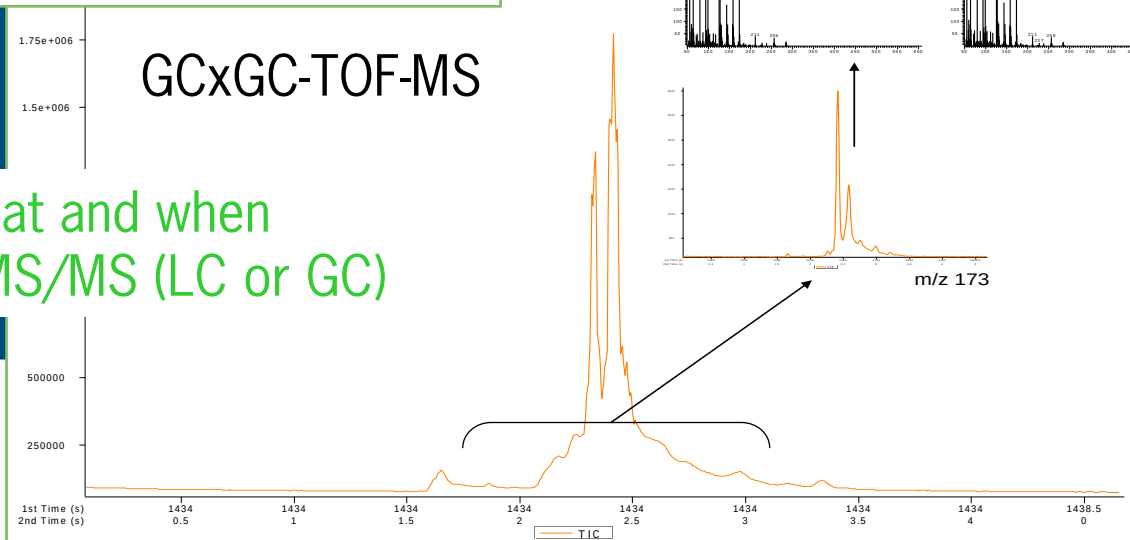
Trivial error

- copy/paste: no
- reporting error: no

Measurement (GC-MS)



Interferences frequently observed for malathion in feed (m/z 173) using GC-MS….



…but less so for wheat and when using GCxGC-MS or MS/MS (LC or GC)

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Calculation

- Standard solution: checked against standard from other laboratory (4-9% diff.)
- Linearity: assigned value within linear range
- Matrix-effect: calibrated using matrix-matched std (CRL control sample)
- Drift: drift: < 10% for subsequent injections of cal. std.
- Calculation error: no

Sample pretreatment

- Malathion recovery data
  - initial validation (compound feed):

    Average REC 62%; RSD 22% (n=5, 0.1 mg/kg)

  - on-going AQC 2008: spikes in different cereals/feed commodities (0.05 mg/kg)

    Results for wheat (n=9):

    Recoveries 51-135%; average 84%

    RSD 30%

=> Analysis lacks robustness but not bad enough to explain bad Z-score:

Good lab performance with -2<Z<+2 => REC 50-150%

?

?????

- Presentation CRL-CF/Mette Poulsen EPRW2008:

  ⇒ water addition prior to extraction results in higher yields
  known also from Fapas PTs, EUPT-C1 (again confirmed)

  ⇒ time between water addition and extraction:
  0 min vs 30 min: no further increase in extraction yield

  ⇒ but: malathion strongly reduced in case of 30 min wetting time

  => Need for more detailed investigation / follow up

1. Literature (search: malathion degradation)

- Hits:

  Yoshii et all, J. Agric. Food Chem. (2000):
  degradation of malathion and phenthoate by glutathione reductase in wheat germ
  Yoshii et all, J. Health Science (2006):
  Malathion residue in wheat kernels is degraded by thion OPP-specific carboxylesterase
  Yoshii et all, J. Health Science (2007):
  Kinetic analysis for hydrolysis of malathion by carboxylesterase in wheat kernels

- findings by Yoshii:

  - carboxylesterase converts malathion into malathion di-carboxylic acid (not malaoxon)
  - only OPP with COOR and P=S are converted (malathion, phenthoate, methacrifos)
  - malathion also converted in oats, barley, rye; but not in corn and rice

  => recommendation: methods should be revised and no water should be added

- contradiction: water needed for efficient extraction but should be avoided to prevent enzymatic conversion

2. Explorative investigations of effect of water

Questions:

- How long do we need to wet ?
- How long do we need to extract ?
- How much water is needed ?

} to maintain the beneficial effect on extraction efficiency

- How to wet the sample and prevent degradation at the same time ?

Enzyme activity:
pH, organic solvent, water content, temp, time

Hydrolysis: pH, temp, time

3. Experiment 1

THANK YOU

CRL-CF

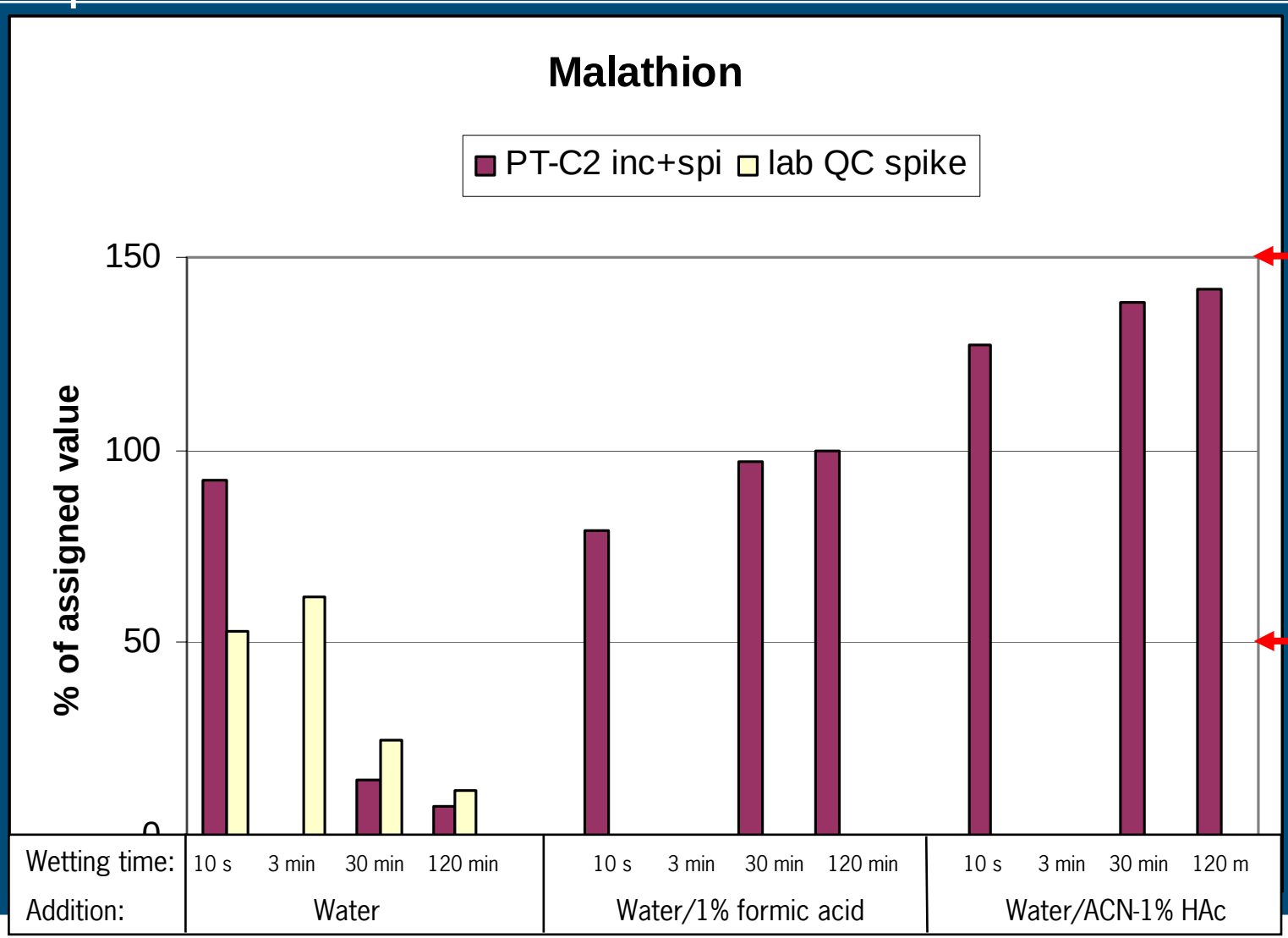
Set up:

- incurred residues (=> wheat EUPT-C1, EUPT-2)
- “classical” Quechers (no PSA) + LC-MS/MS
- matrix-matched calibration
- test set:

sample	2.5 g wheat (CRL PT samples 2007-2008)		
wetting	7.5 ml water	7.5 ml water/1% FA	17.5 ml water/ACN/1% HAc
static wetting time	10 s 3 min 30 min 120 min	10 s 30 min 120 min	10 s 3 min 30 min 120 min
solvent	10 ml ACN/1% HAc	10 ml ACN/1% HAc	-
addition of salts	4 g MgSO4/1 g NaAc	4 g MgSO4/1 g NaAc	-
vortex/shake	3 min	3 min	3 min
addition of salts	-	-	4 g MgSO4/1 g NaAc
vortex/shake	-	-	30 s

- remark: only single analysis, different days

3. Experiment 1: Results



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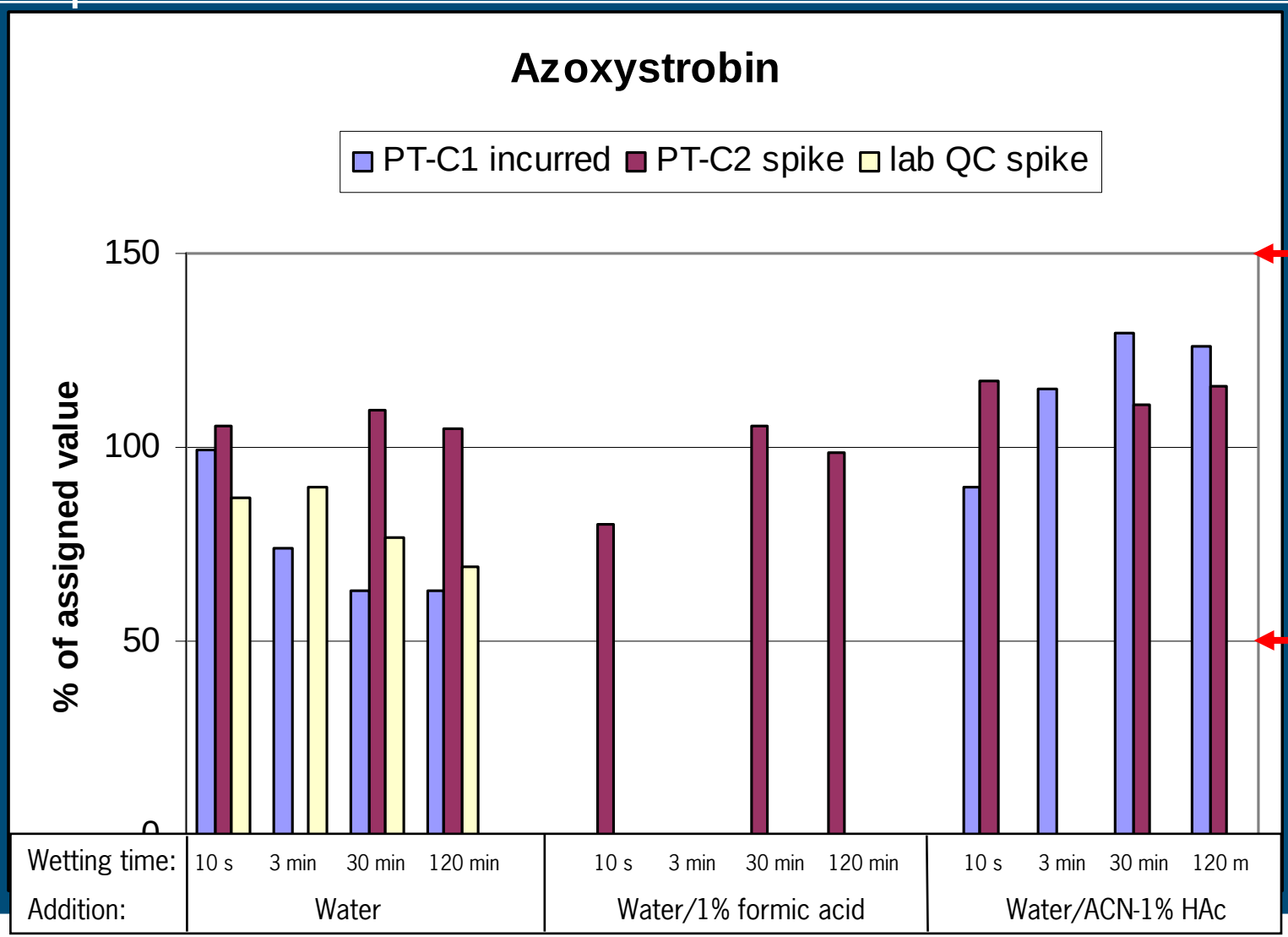
⇒ water + > 5-10 min wetting time: unacceptable results
⇒ acidification inhibits degradation
⇒ water/ACN/acid highest results (pos bias)

3. Experiment 1: Results



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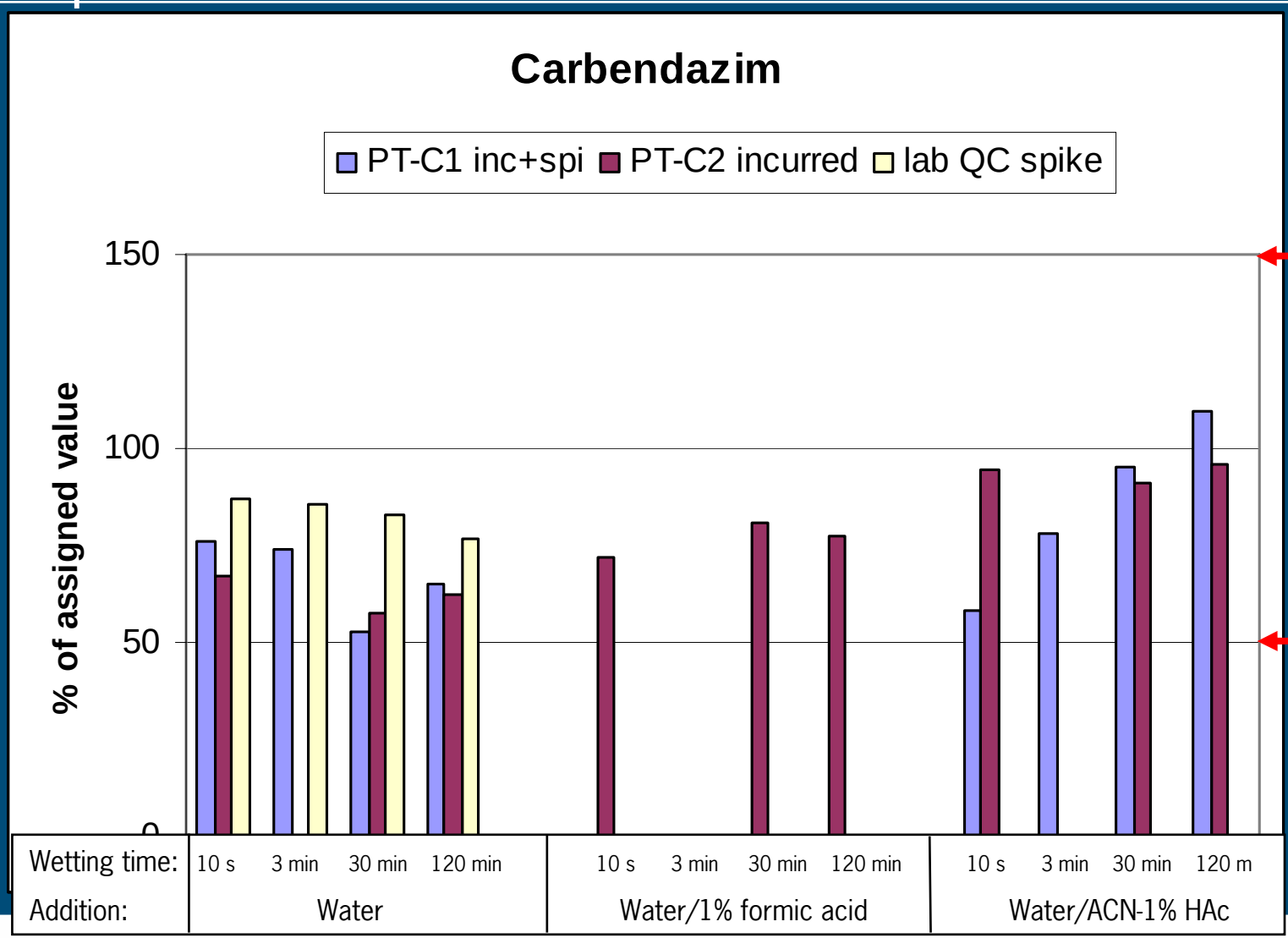
⇒ Adequate performance in all cases

⇒ water/longer wetting time: neg bias; water/ACN/acid: pos bias

3. Experiment 1: Results



⇒ Adequate performance in all cases
⇒ water/longer wetting time: lowest values;
⇒ water/ACN/acid longer wetting time: highest values

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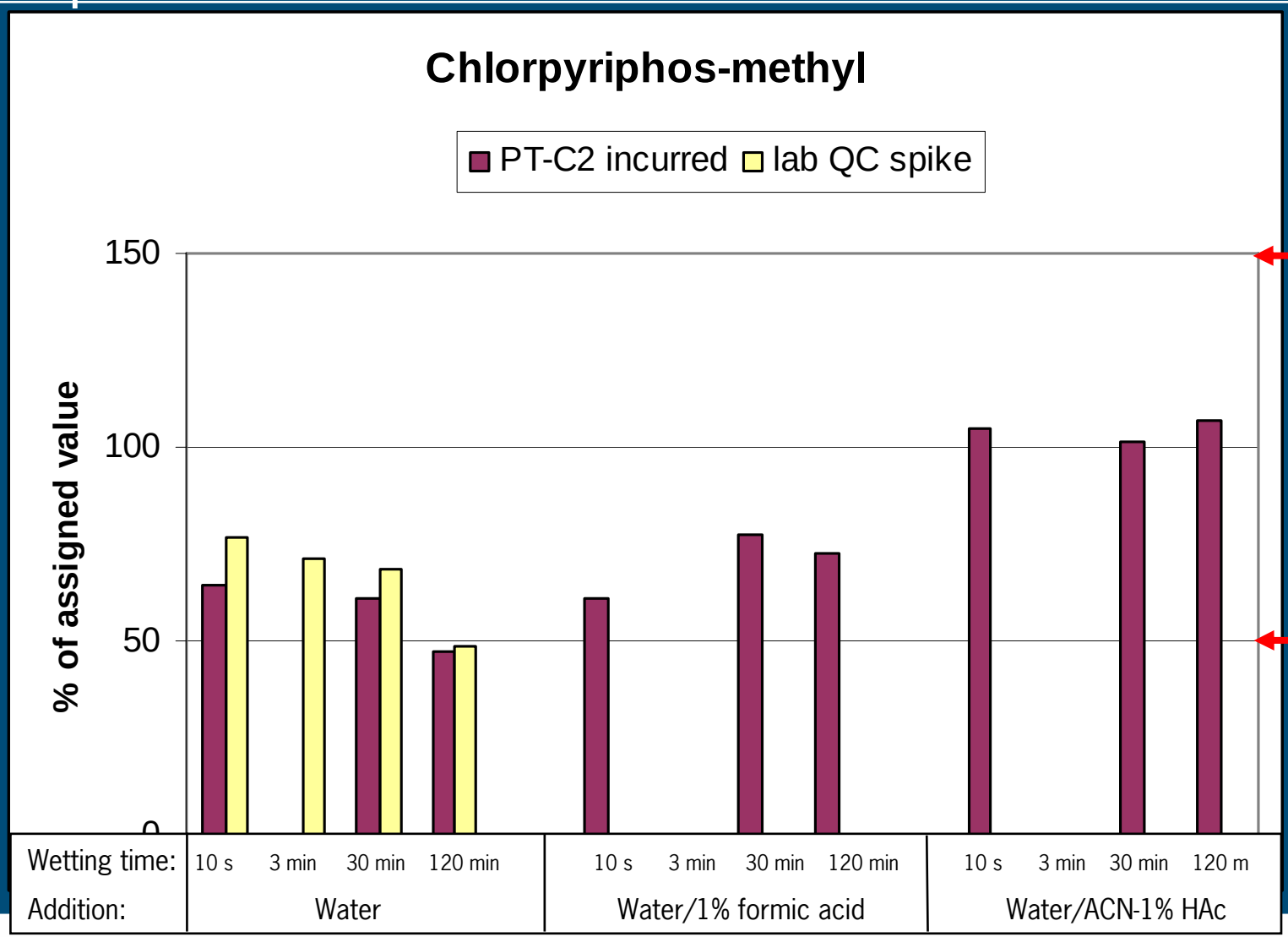
3. Experiment 1: Results



⇒ Adequate performance in all cases, except water/120 min
⇒ (acidified) water: neg bias
⇒ water/ACN/acid: highest values

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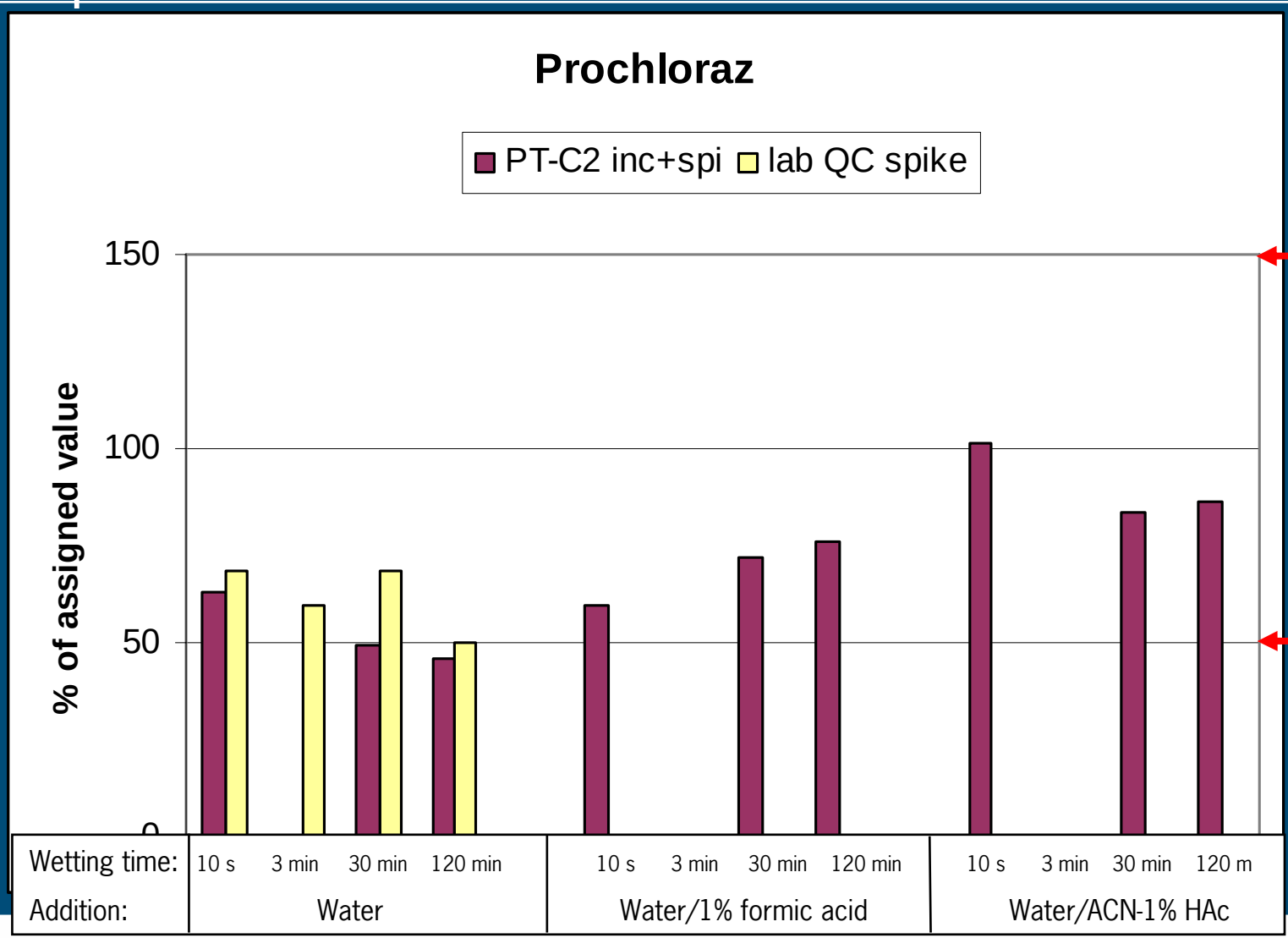
3. Experiment 1: Results



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⇒ Adequate performance in all cases, except water ≥ 30 min

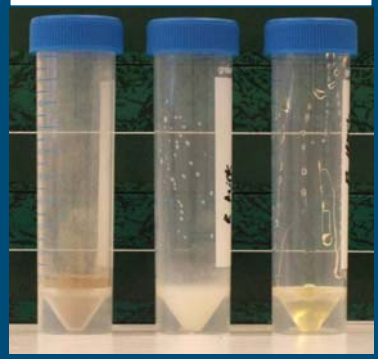
3. Experiment 1: tentative conclusions

- Differences are observed when comparing different procedures, but only in few cases this results in unacceptable Z-scores
- Most of the residues in wheat flour stable for > year (freezer)
- Prolonged wetting with water can result in lower values/neg bias
- Acidification of water added prior to extraction inhibits degradation (malathion)
- Water addition prior to extraction vs addition of mixture water/ACN: there is no need to add water first and then acetonitrile; simultaneous wetting/extraction gives highest values/pos bias

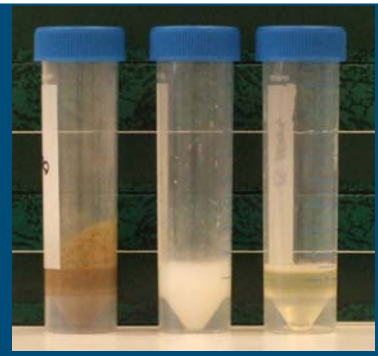
⇒ Optimum initial extraction very similar to generic extraction procedure presented at EPRW2008 (Mol et all)

Generic extraction method (pest/nat. toxins/vet. drugs)

1) take 2.5 g

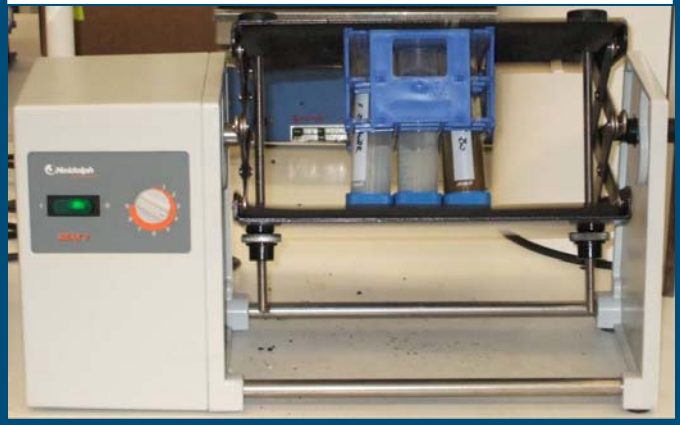


2) add 20 ml extr. solvent*

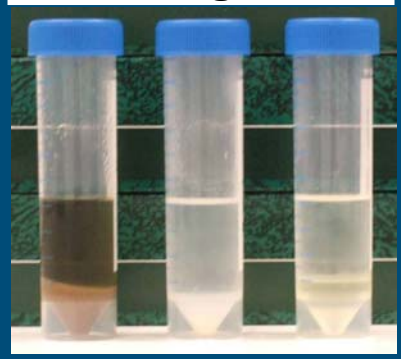


* water/ACN/FA (25/75/1)

3) shake 1 h (arbitrarily chosen)



4) Centrifuge



5) Inject into UPLC-MS/MS

3. Experiment 2

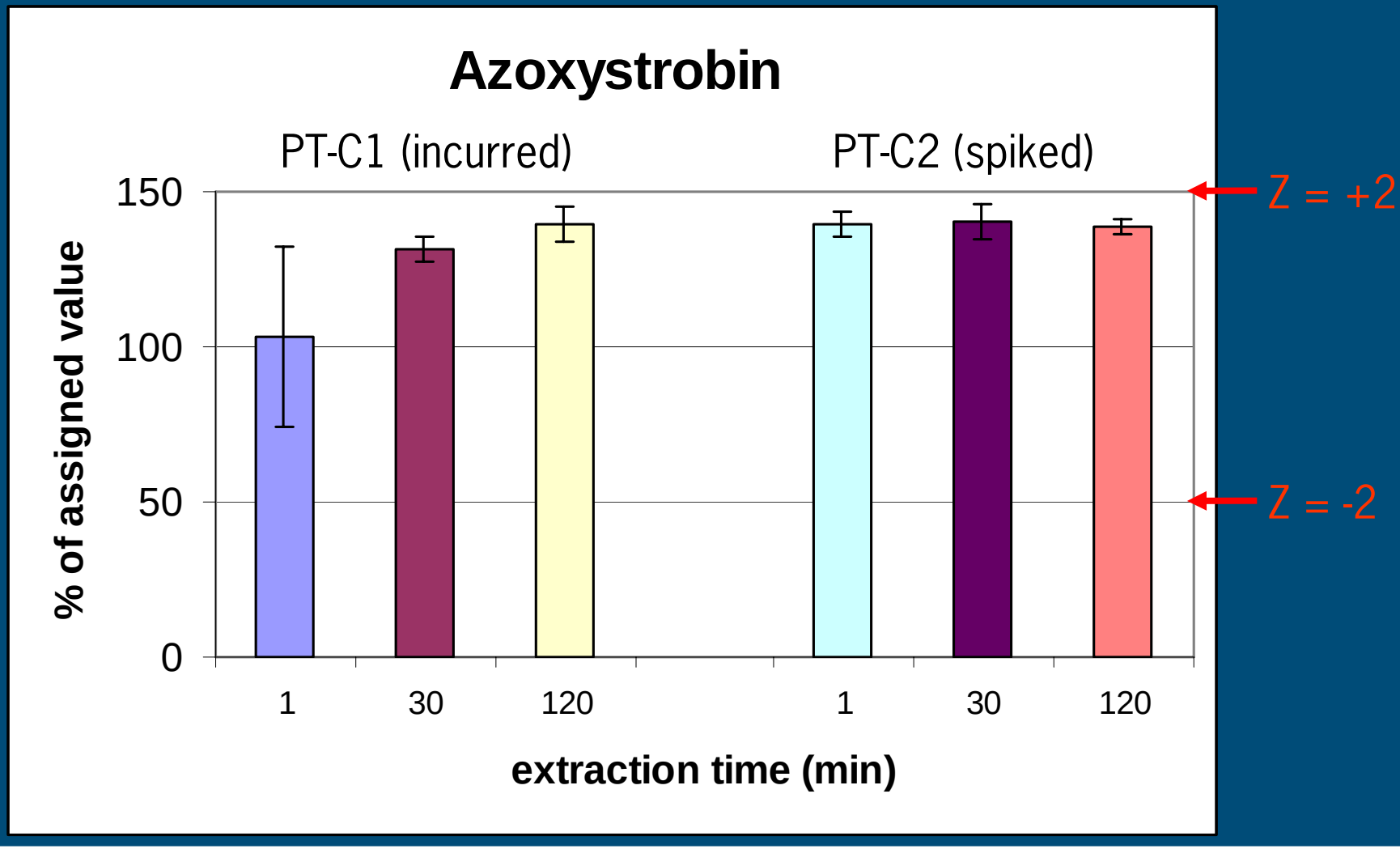
Goals:

- test extraction efficiency of generic method using samples with incurred residues
- establish required wetting/extraction time

Set up:

- wheat EUPT-C1, EUPT-2
- 2.5 g sample; 10 ml extraction solvent (water/ACN/FA 25/75/1)
- shaking 1 min (hand/vortex); 30 min; 120 min (machine) [triplicates]
- no salts/partitioning; centrifugation and injection into LC-MS/MS
- matrix-matched calibration

3. Experiment 2: Results



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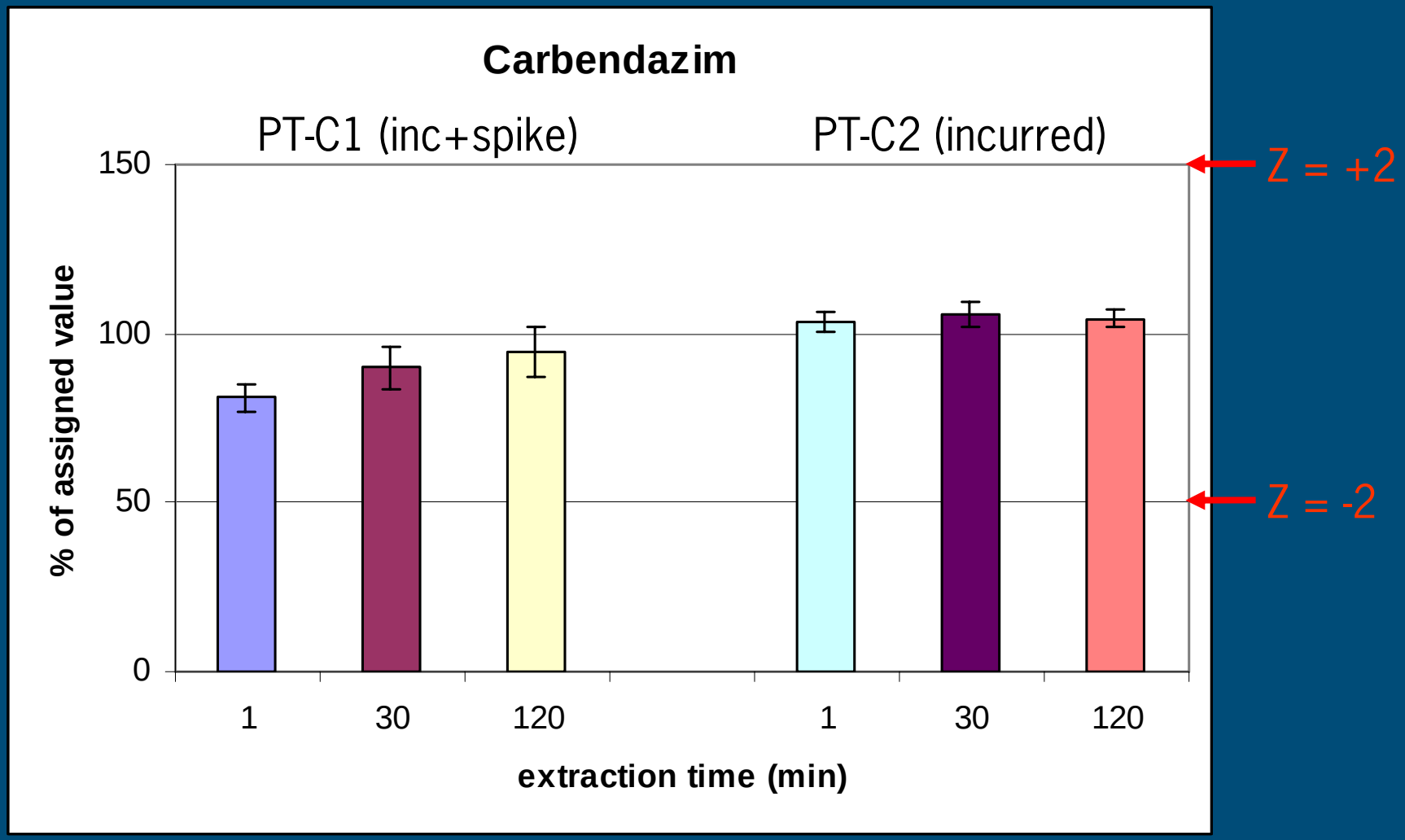

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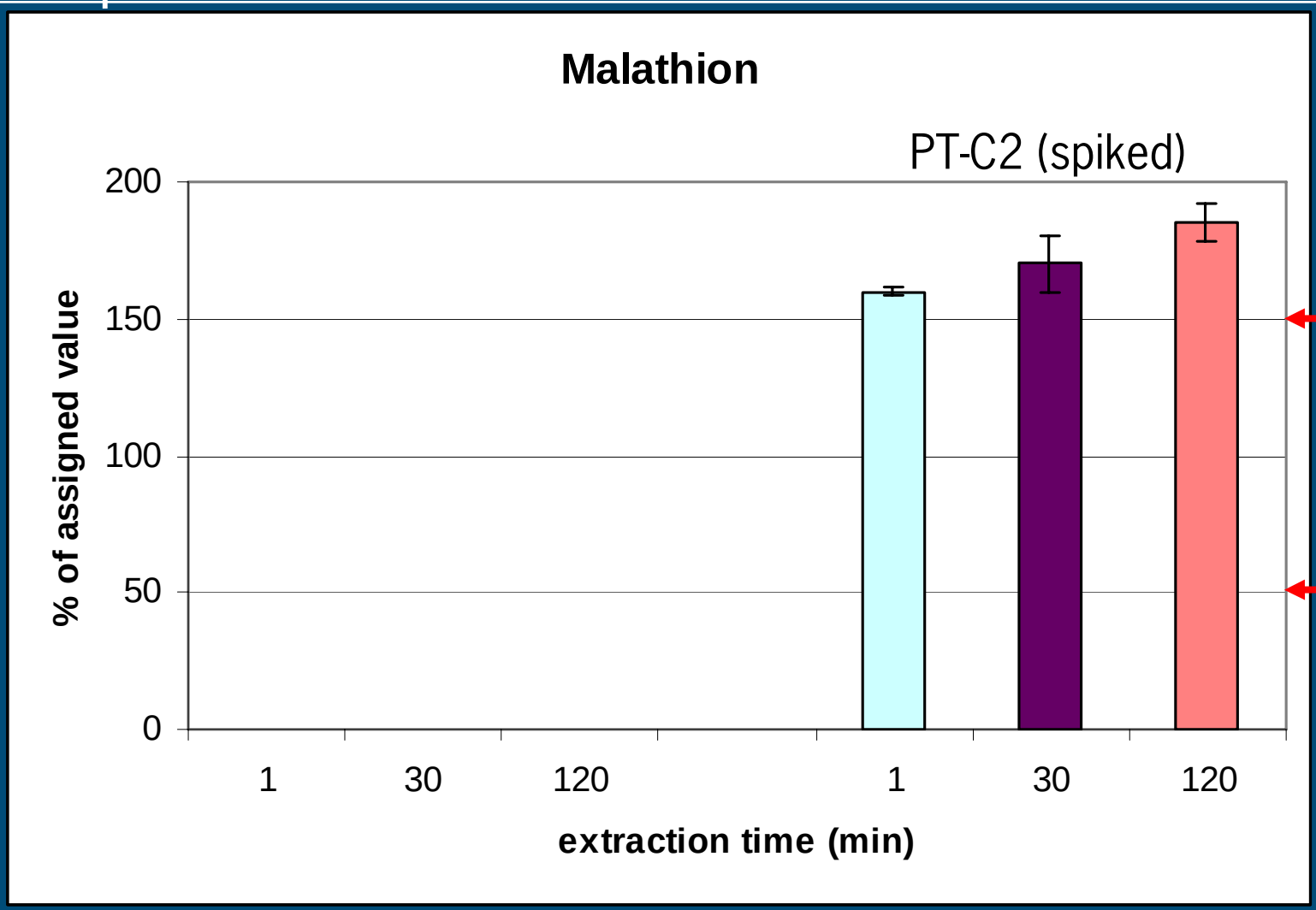

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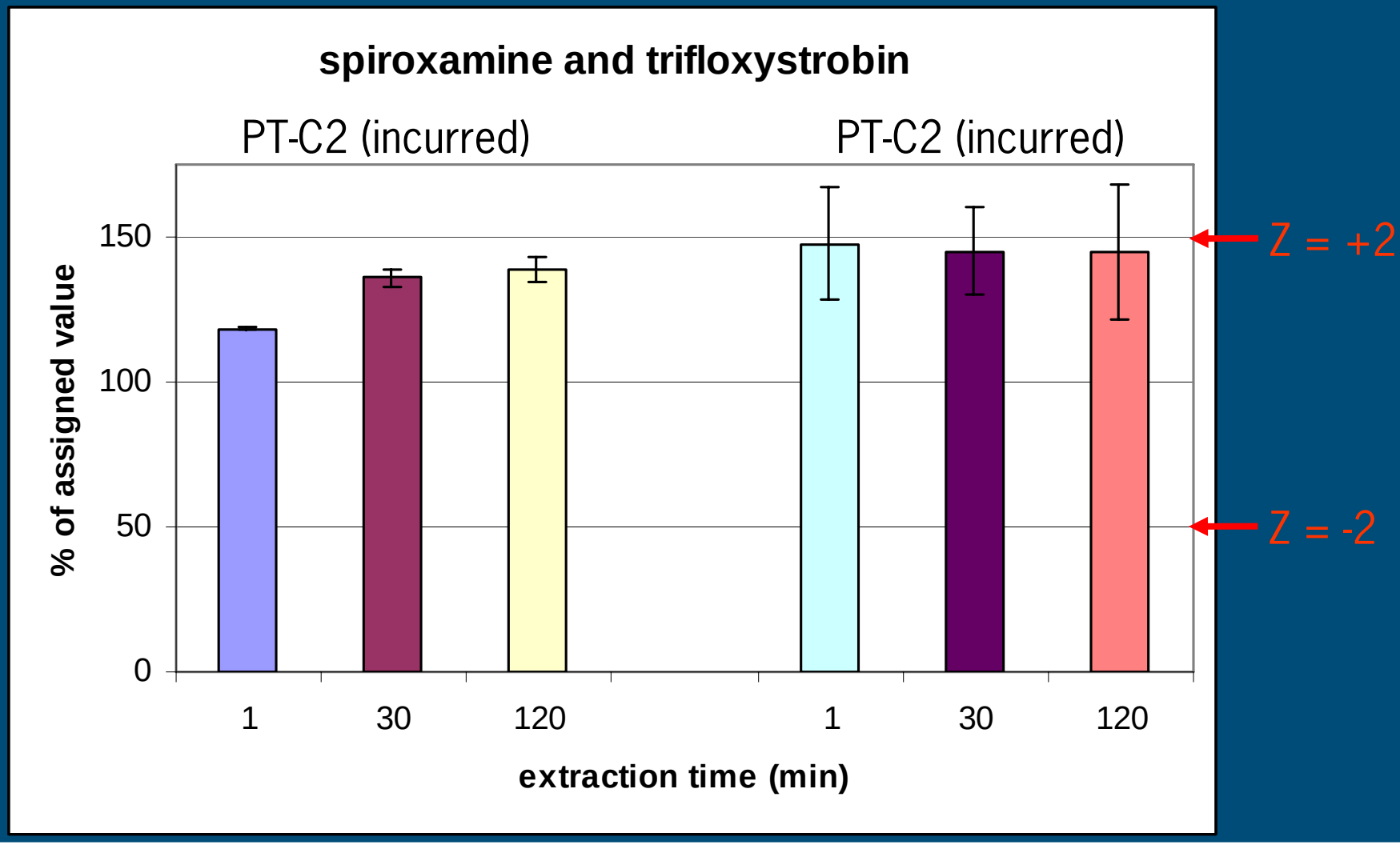

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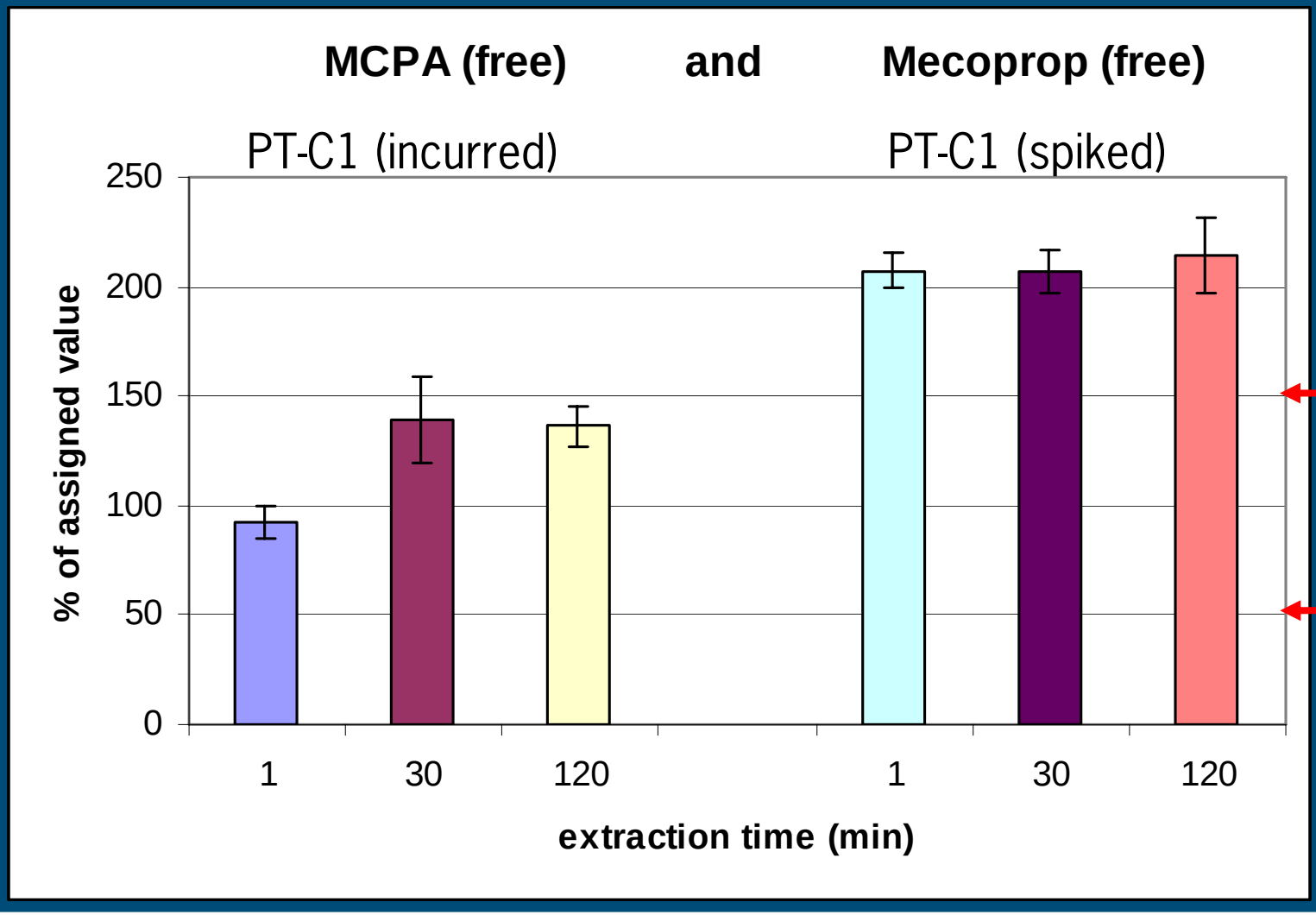


3. Experiment 2: conclusions

- 1 min extraction time is not sufficient in all cases (significant?)
- beyond that: extraction time has little effect / is not a critical parameter
  - no increase (improvement in extraction efficiency)
  - no decrease (degradation of pesticides)
- Extraction with water/ACN/FA (25/75/1) is efficient and robust
- Results for malathion now significant higher then assigned value (Z>2) !

3. Experiment 3

How much water is needed for efficient extraction ?

- acetonitrile based method
  % water, ratio of water and solvent to sample

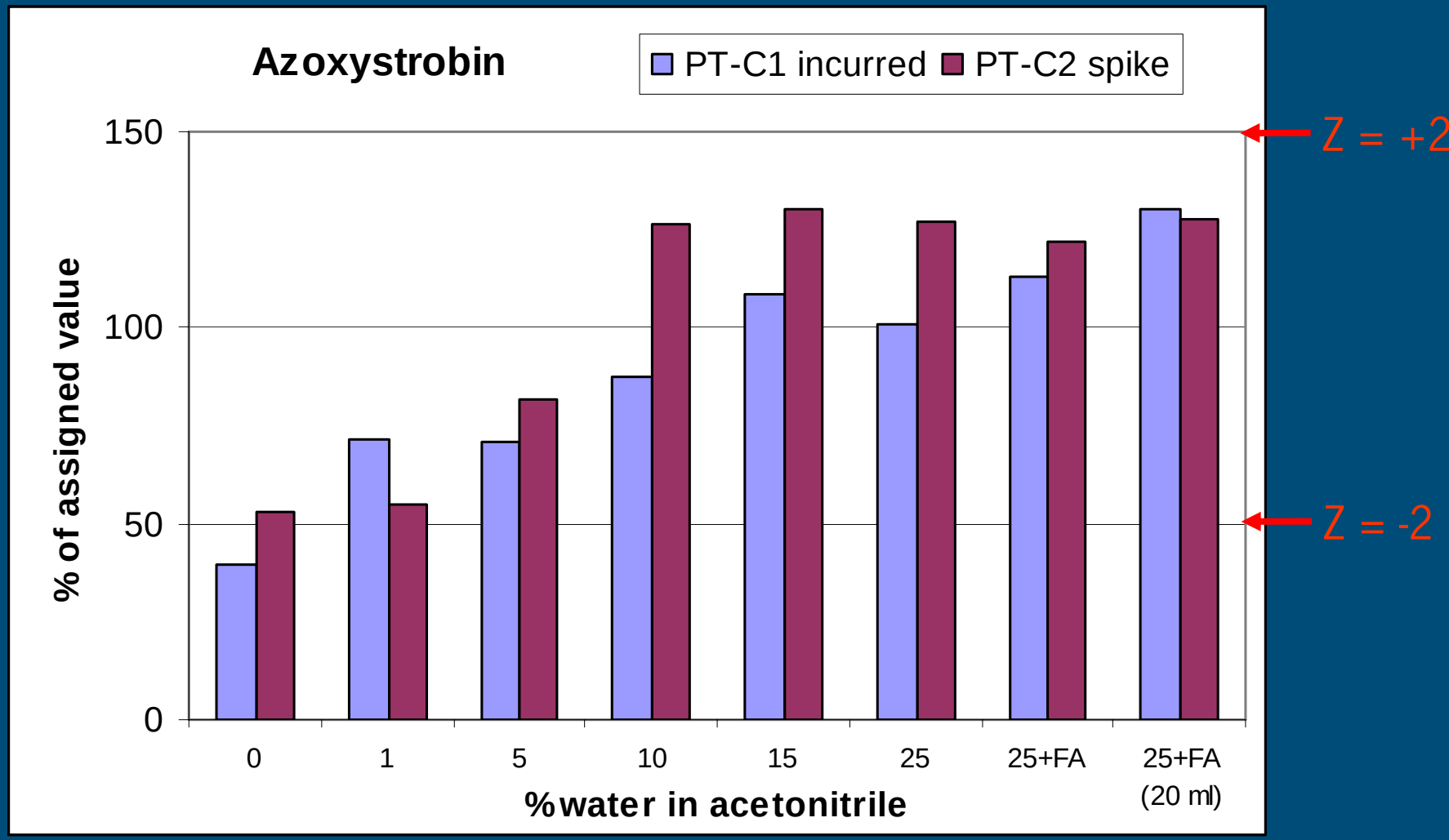
	extraction solvent			ratio	ratio
sample	volume	acetonitrile	water	water:sample	solvent:sample
2.5 g EUPT-C	10	100	0	>>	4
2.5 g EUPT-C	10	99	1	0.004	4
2.5 g EUPT-C	10	95	5	0.2	4
2.5 g EUPT-C	10	90	10	0.4	4
2.5 g EUPT-C	10	85	15	0.6	4
2.5 g EUPT-C	10	75	25	1	4
2.5 g EUPT-C	10*	75	25	1	4
2.5 g EUPT-C	**20***	75	25	2	8
	* containing 1% FA				

Extraction time 30 min

Matrix-matched calibr.

Duplicate analysis

3. Experiment 3: Results



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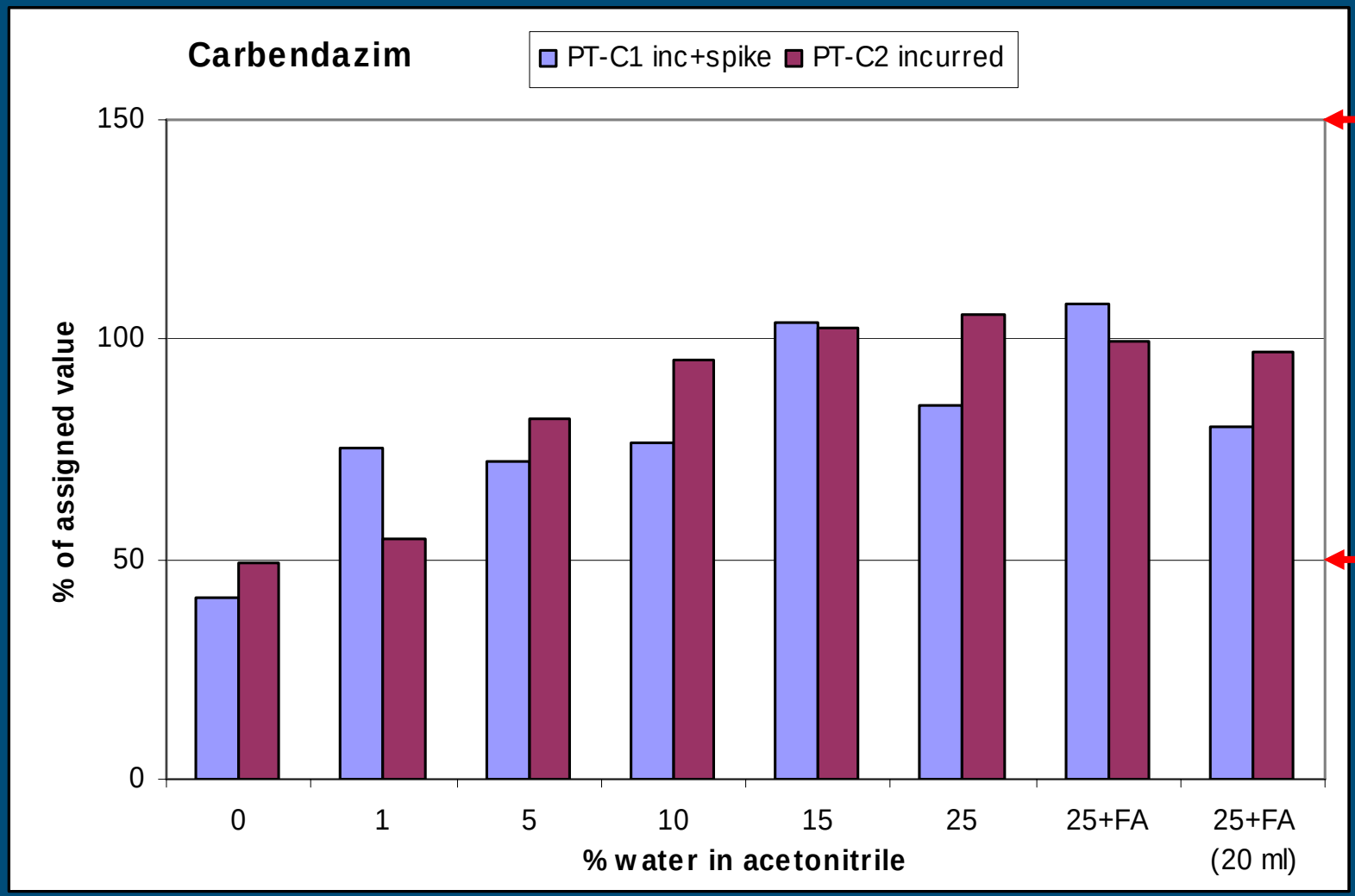
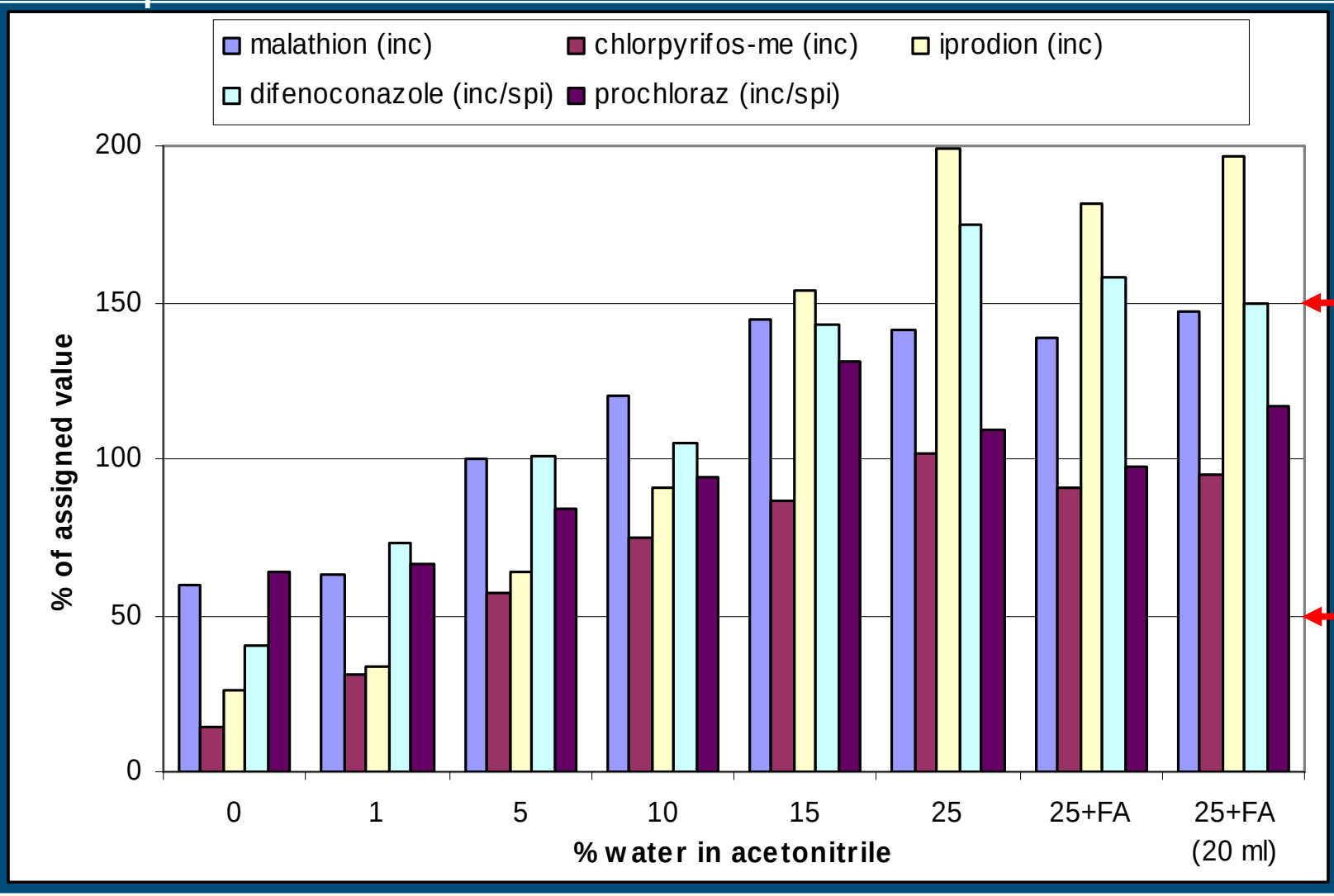


3. Experiment 3: Results



3. Experiment 3: conclusions

- at least 15% water in acetonitrile (10 ml) = [1.5 ml per 2.5 g sample] is required for efficient extraction of several pesticides from wheat flour
- more water does not further increase extraction efficiency
- for some pesticides (iprodion, difenoconazole) values significantly higher than the assigned value are obtained

Comparison original results with results new method

	pesticide	residue	assigned µg/kg	method		reported µg/kg	Z-score	method		set-1 av (n=3)	calc. Z-score	set-2 av (n=2)	calc. Z-score
EUPT-C1 2007	Azoxystrobin	inc	240	ACN	LC	234	-0.1	generic	LC	316	**1.3**	272	0.5
	Carbendazim	inc/spi	126	ACN	LC	113	-0.4	generic	LC	113	-0.4	136	0.3
	Deltamethrin	inc/spi	342	EtAc	**GC**	436	**1.1**						
	Diazinon	inc	78	ACN	LC	87	0.5	generic	LC	76	-0.1	95	0.9
	Pirimiphos-methyl	inc	6330	ACN	LC	7260	0.6						
	Propiconazole	spi	353	ACN	LC	314	-0.4	generic	LC	518	**1.9**	484	**1.5**
	Endosulfan	inc	n.a.	EtAc	**GC**	35	n.a.						
EUPT-C2 2008	Azoxystrobin	spi	239	EtAc	LC	234	-0.1	generic	LC	334	**1.6**	291	0.9
	Bifenthrin	inc	87	EtAc	**GC**	94	0.3						
	Carbendazim	inc	570	EtAc	LC	709	1.0	generic	LC	602	0.2	568	0.0
	Chlorpyrifos-methyl	inc	130	EtAc	**GC**	141	0.3	generic	LC	118	-0.4	118	-0.4
	Cypermethrin	inc	98	EtAc	**GC**	76	-0.9						
	Difenoconazole	inc/spi	169	EtAc	LC	171	0.0	generic	LC	250	**1.9**	266	**2.3**
	Epoxiconazole	inc	176	EtAc	LC	187	0.3						
	Iprodione	inc	289	EtAc	LC	355	0.9	generic	LC	623	**4.6**	525	**3.3**
	Malathion	inc/spi	162	EtAc	**GC**	< 50 (0.034)	**-4.0**	generic	LC	275	**2.8**	224	**1.5**
	Pirimicarb	inc	38	EtAc	LC	39	0.2	generic	LC	49	**1.2**	44	0.6
	Prochloraz	inc/spi	239	EtAc	LC	267	0.5	generic	LC	240	0.0	233	-0.1
	Spiroxamine	inc	75	EtAc	LC	54	**-1.1**	generic	LC	102	**1.4**	118	**2.3**
	Trifloxystrobin	inc	439	EtAc	LC	432	-0.1	generic	LC	638	**1.8**	562	**1.1**

?? Improvement or not ??

4. Conclusions

- The reason for the bad Z-score for malathion has been found (enzym. degr.)
- Differences are observed in extraction efficiency of spiked and incurred residues
  Recoveries from spiked AQC samples do not always reflect reality
- Need for addition of water to low-moisture matrices again confirmed
- Exposure time of sample to water prior to extraction is critical parameter
  SOPs adjusted: EtAc method: minimize wetting time
  ACN (Quechers): add mixture of water/ACN
- With water/ACN (FA) mixtures, extraction time is not a critical parameter
- With water/ACN (FA) mixtures, ratio water:sample should be at least 3:5
- For several pesticides, extraction with water/ACN (FA) mixtures increased levels of pesticdes were found (clearly above the assigned values:
  propiconazole, difenoconazole, iprodion, malathion, spiroxamine?, trifloxystrobin?)
- a 1-to-1 comparison of the different methods employed needs to be performed to confirm findings and establish significance of differences in extraction efficiency

Acknowledgement

CRL-CF people

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Theo de Rijk

Darinka Stajnbaher (Public Health Institute, Maribor, Slovenia)

Thank you for your attention

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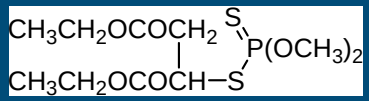
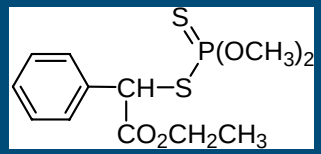
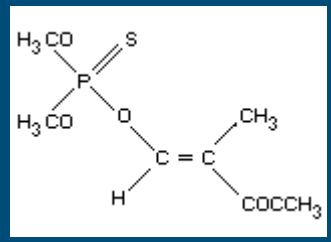
Structures OPs



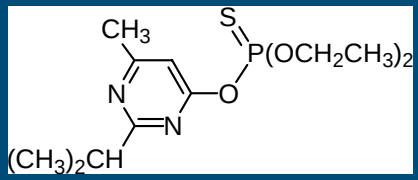
Malathion



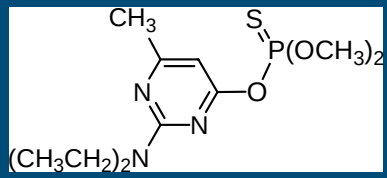
phenthoate



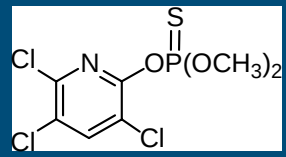
methacrifos



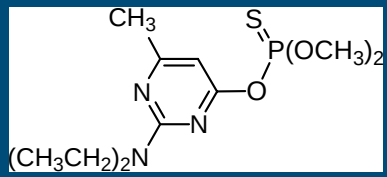
diazinon



Pirimiphos-methyl



Chlorpyrifos-methyl



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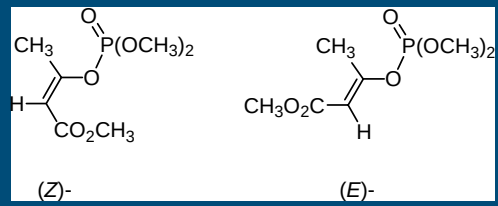

mevinphos

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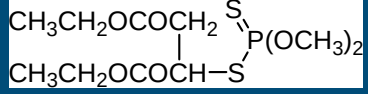

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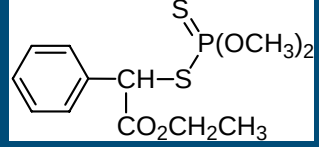

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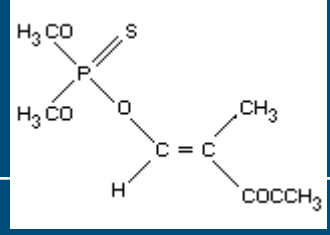

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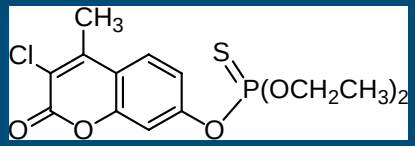

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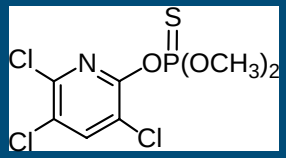
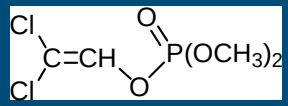


Chlorpyrifos-methyl



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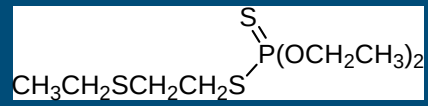

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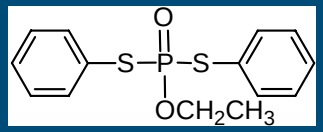
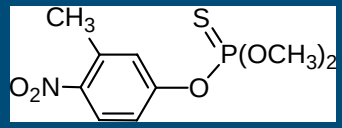


Edifenphos



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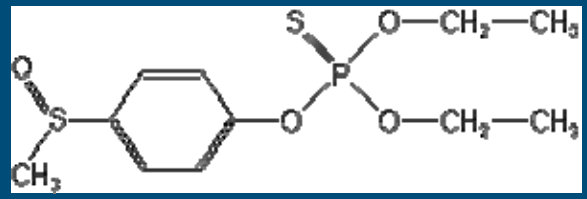

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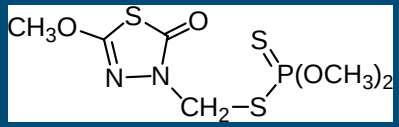

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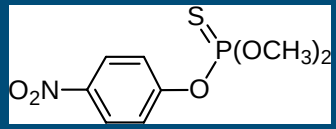
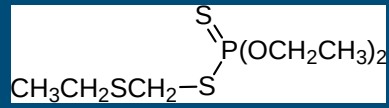
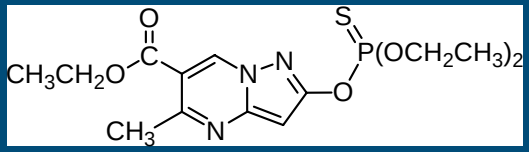


Parathion-methyl



Phorate



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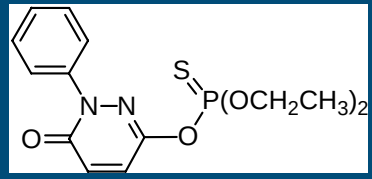

pyridaphenthion

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