Co-extracted compounds from cereals and parameters affecting the extraction and clean-up efficiency of incurred pesticide residues

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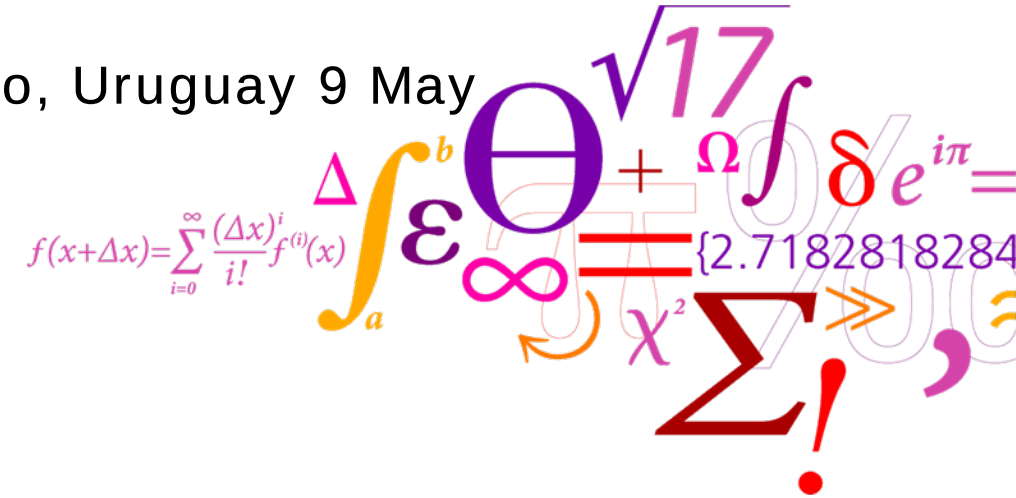


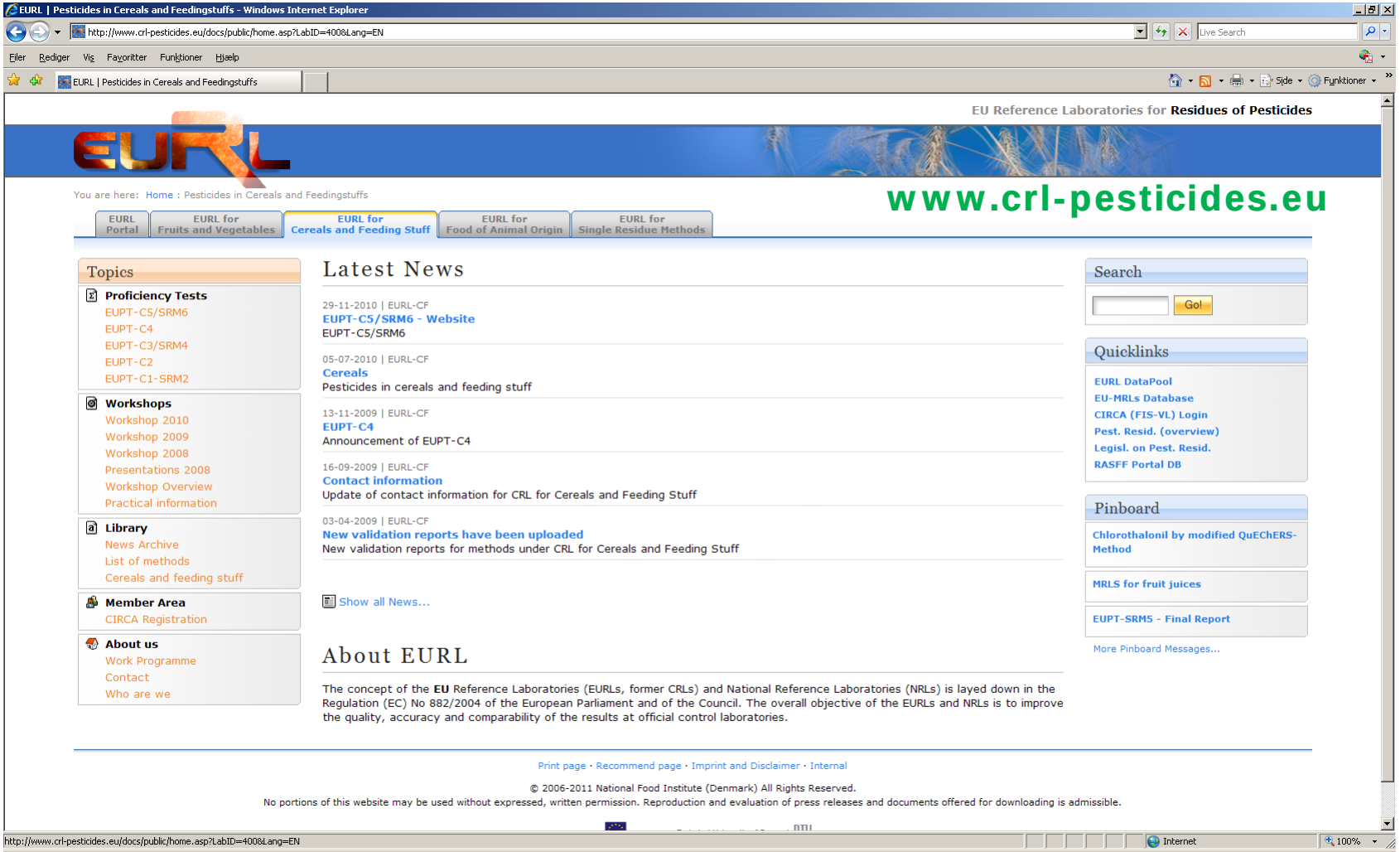
DTU Food
National Food Institute

Agenda

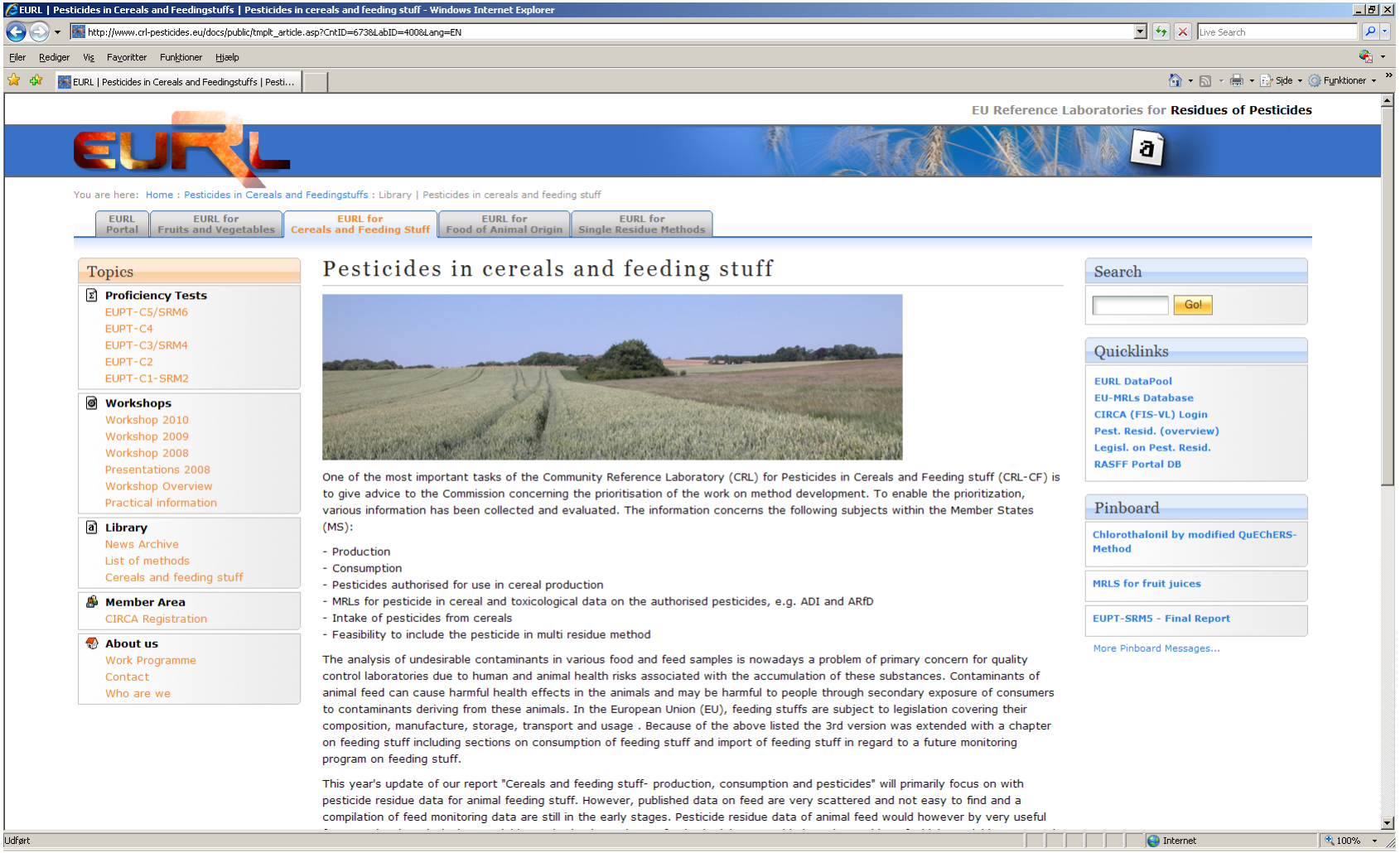
- EURL for cereals and feeding stuff
- Co-extracted compound
  - Different matrices, solvent, freezing step, clean-up with PSA, storage of samples
- Extraction efficiency experiments
  - Water addition, solvents
- Results from EUPT-C4
  - Multi variable statistical treatment.
- Conclusions

National Food Institute, Technical University of Denmark

EU reference laboratories for pesticide residues - web portal



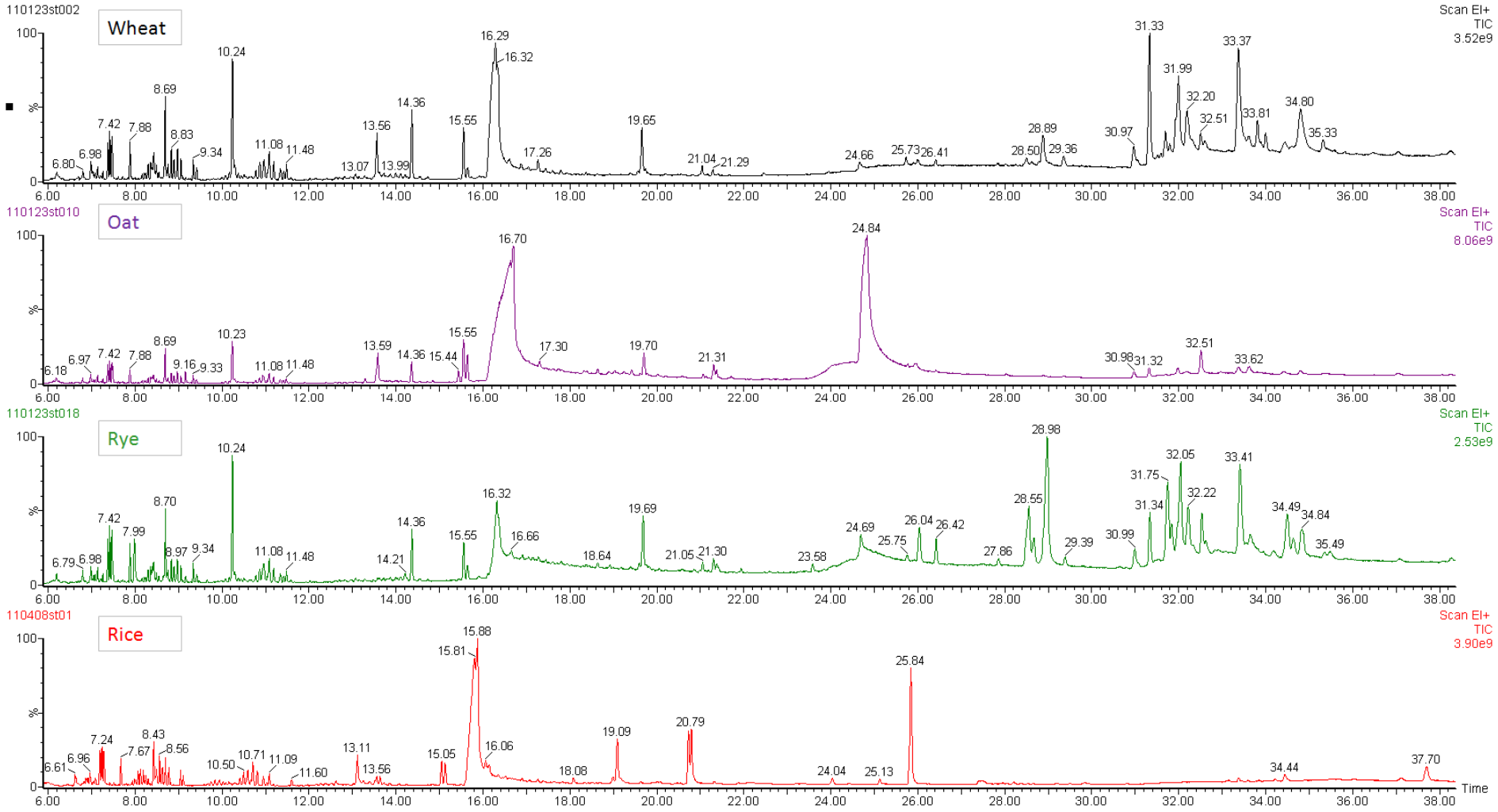
EU reference laboratories for pesticide residues - web portal

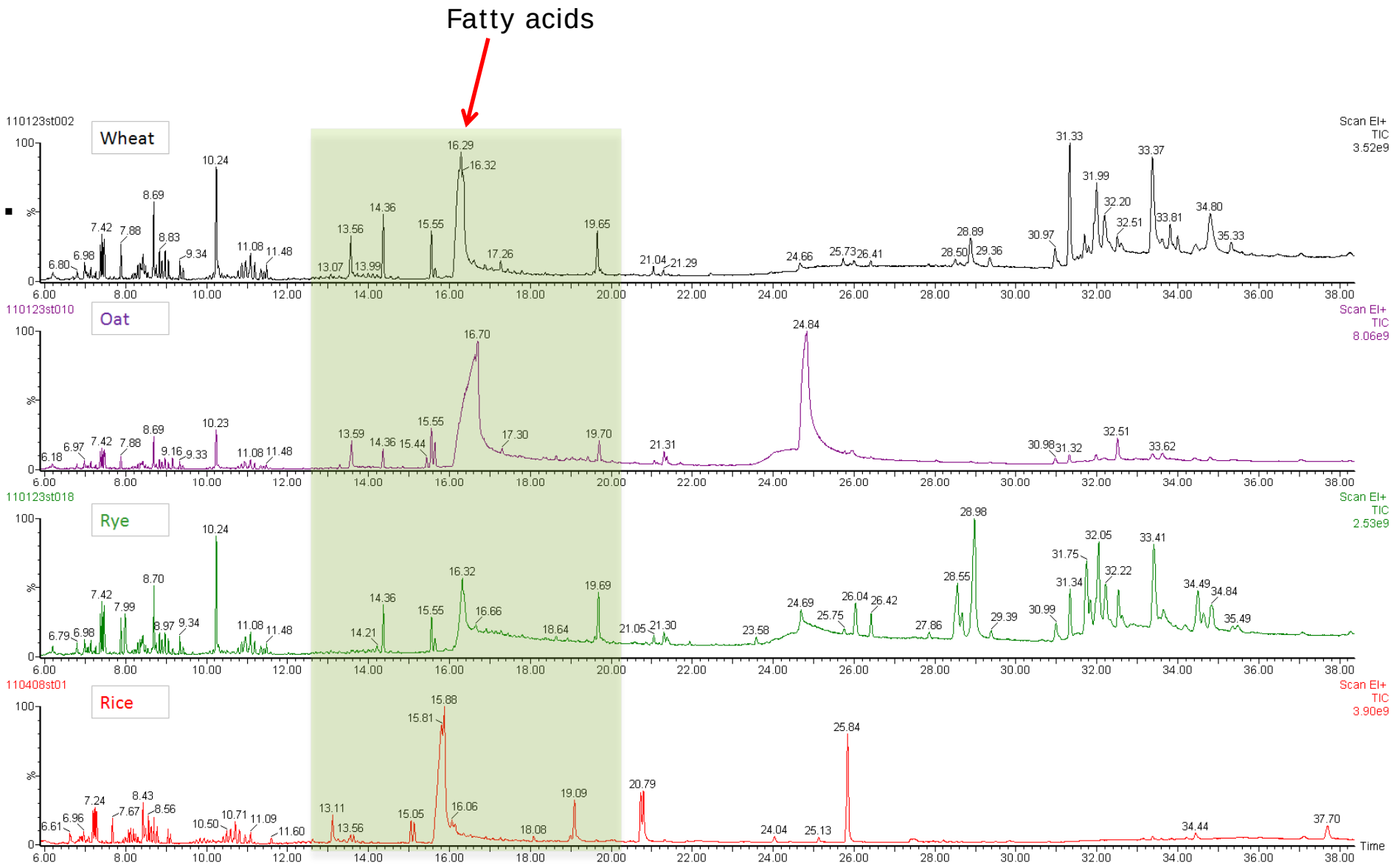


EUPTs / cereals – overview

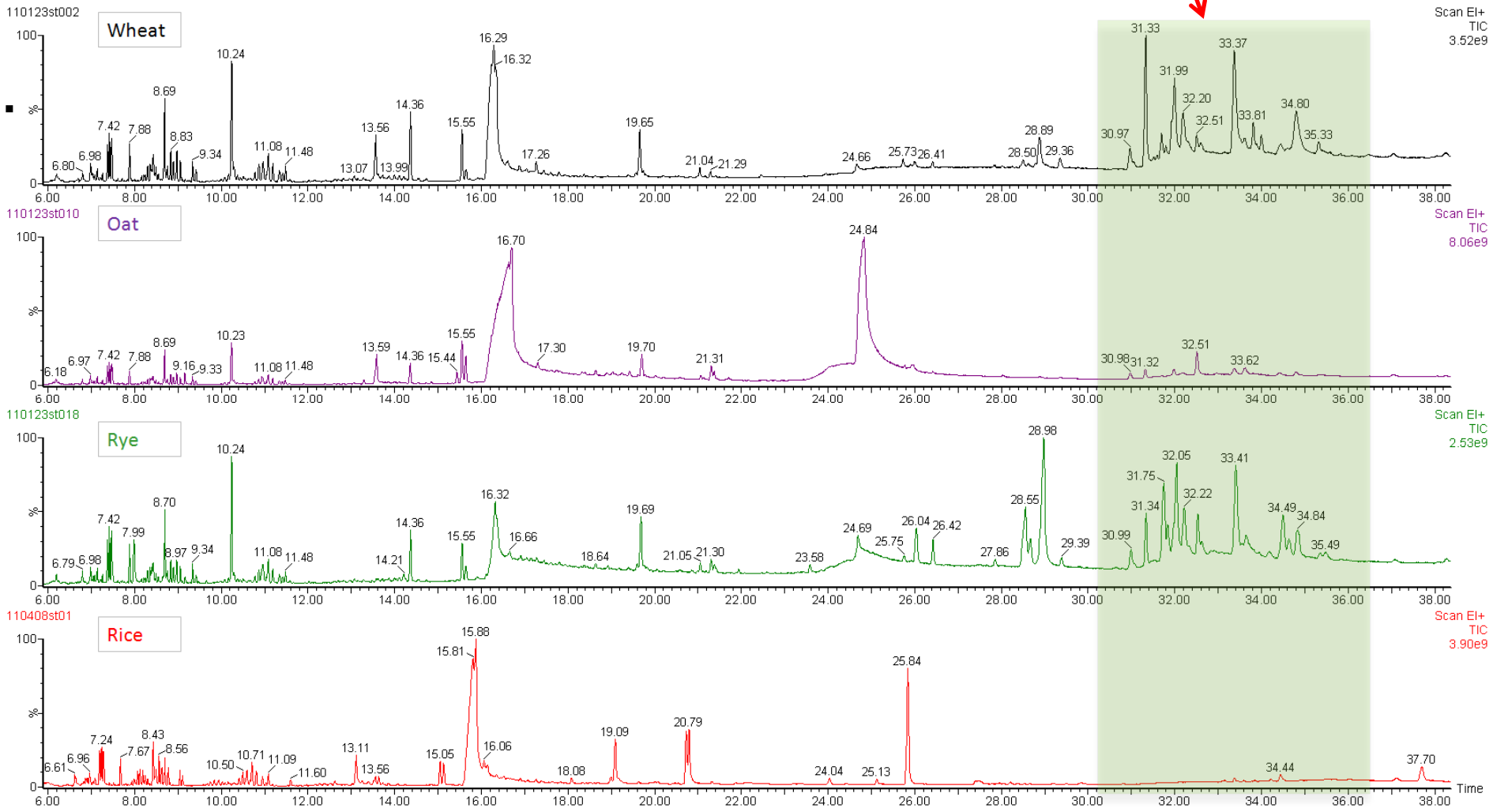
	2007 EUPT-C1/SRM2	2008 EUPT-C2	2009 EUPT-C3/SRM4	2010 EUPT-C4	2011 EUPT-C5/SRM6
Test material	**Wheat flour**	**Wheat flour**	**Oat flour**	**Rye flour**	**Rice flour**
no. of participants (EU)	64	74	111	118	135
no. of target pesticides	**37**	**55**	**60**	**70**	**103**
no. of incurred pesticides MRM	**3**	**9**	**14**	**13**	**10**
no. of spiked pesticides MRM	**4**	**4**		**3**	**7**
no. of incurred pesticides SRM	**2**	**2**	**2**	**2**	
no. of spiked pesticides SRM	**1**		**2**	**1**	**7**
Total no. of pesticides in test ma	**10**	**15**	**18**	**19**	**24**

Coextracted compounds from cereals Quechers method – GC/MS scan mode




Fatty acids
110123st002
Wheat
Scan EI+
TIC
3.52e9
110123st010
Oat
Scan EI+
TIC
8.06e9
110123st018
Rye
Scan EI+
TIC
2.53e9
110408st01
Rice
Scan EI+
TIC
3.90e9
%
Time

Phytosterols

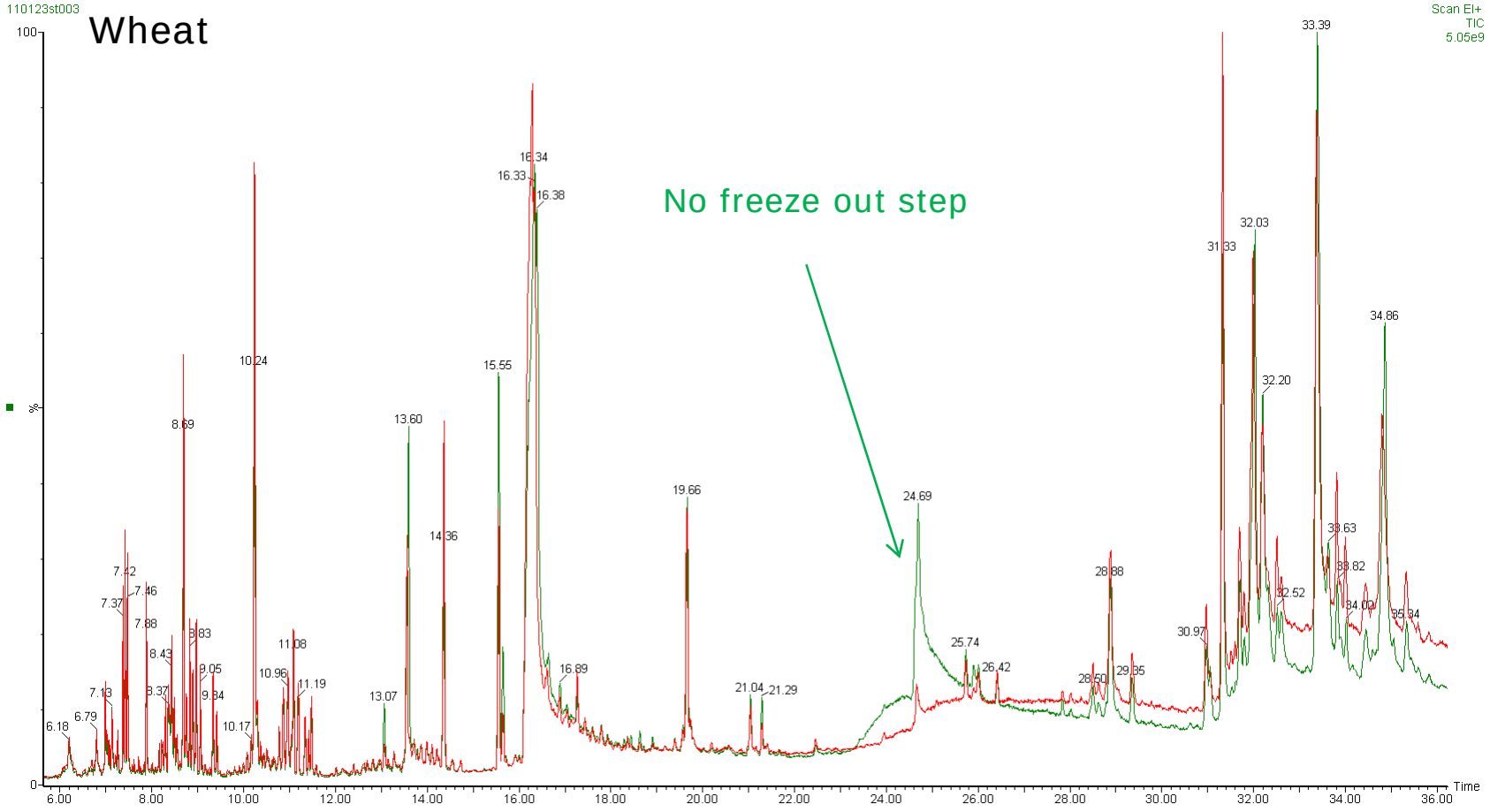


Freeze out step, -80 °C in one hour

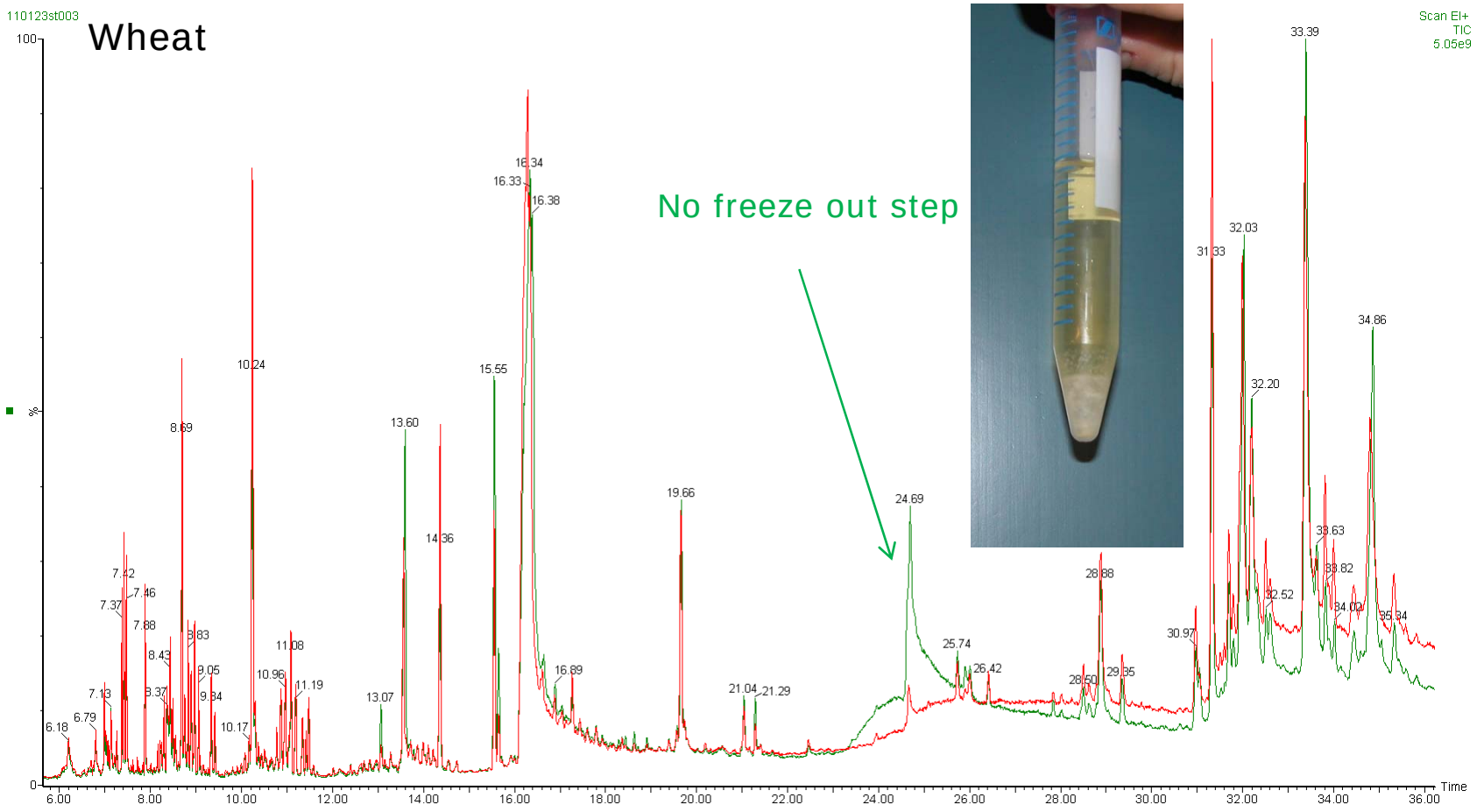
- 8 ml af raw extract of Quechers method are transfered to a 15 ml tube and put in a -80 °C freezer for one hour.

- Centrifugation at in cold centrifuge for 5 min

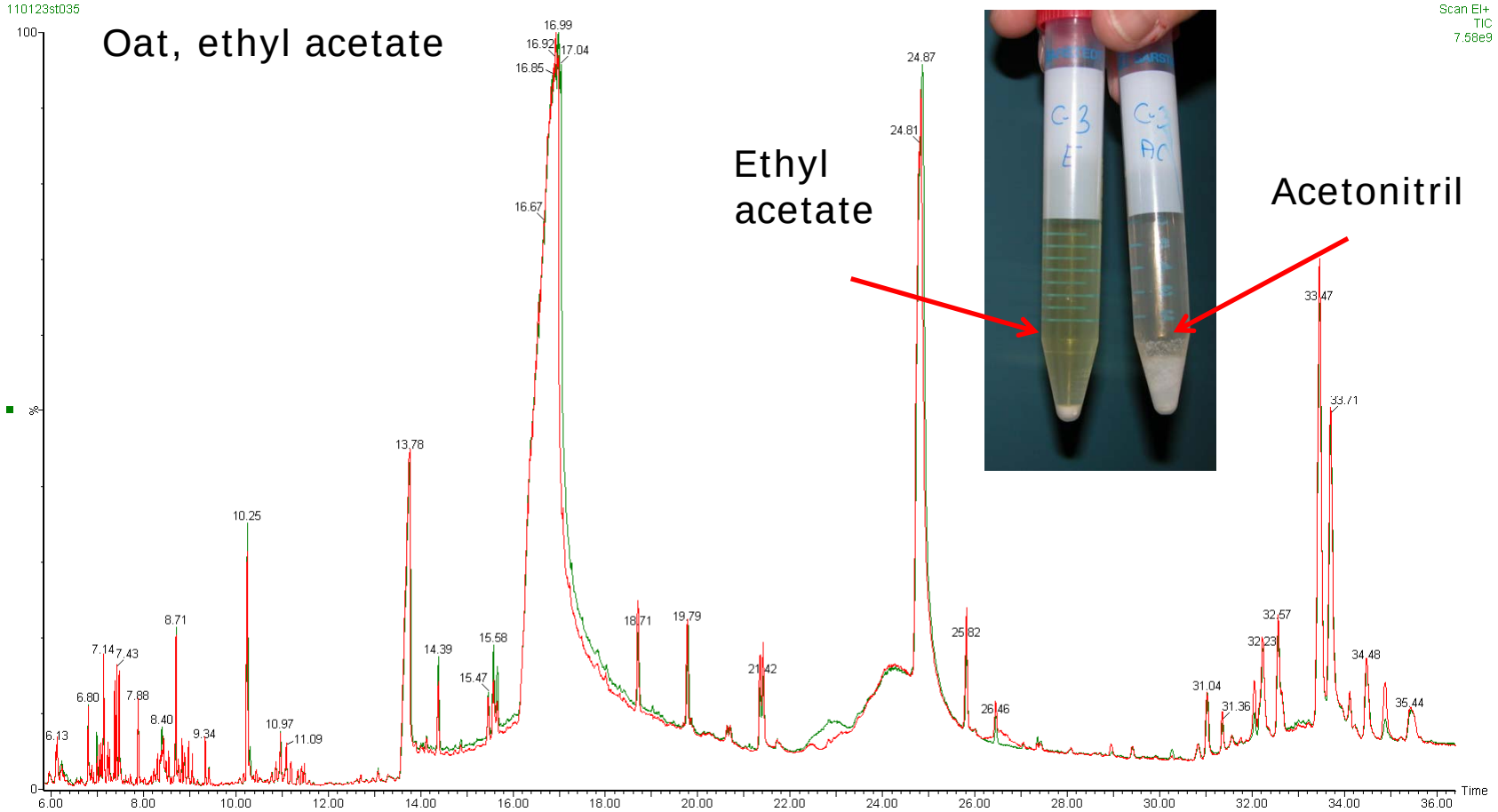
Freeze out step, -80 °C in one hour



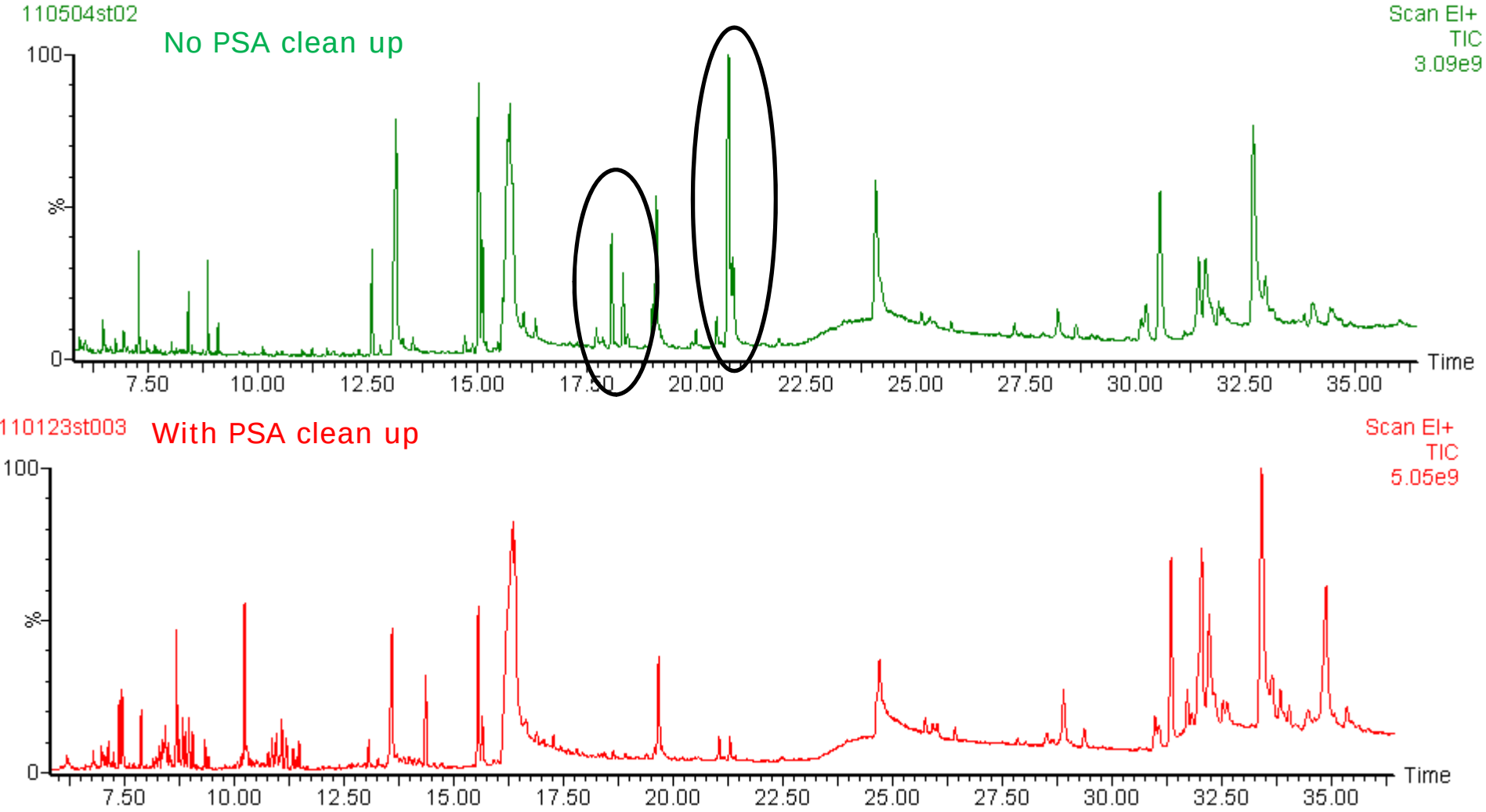
Freeze out step, -80 °C in one hour



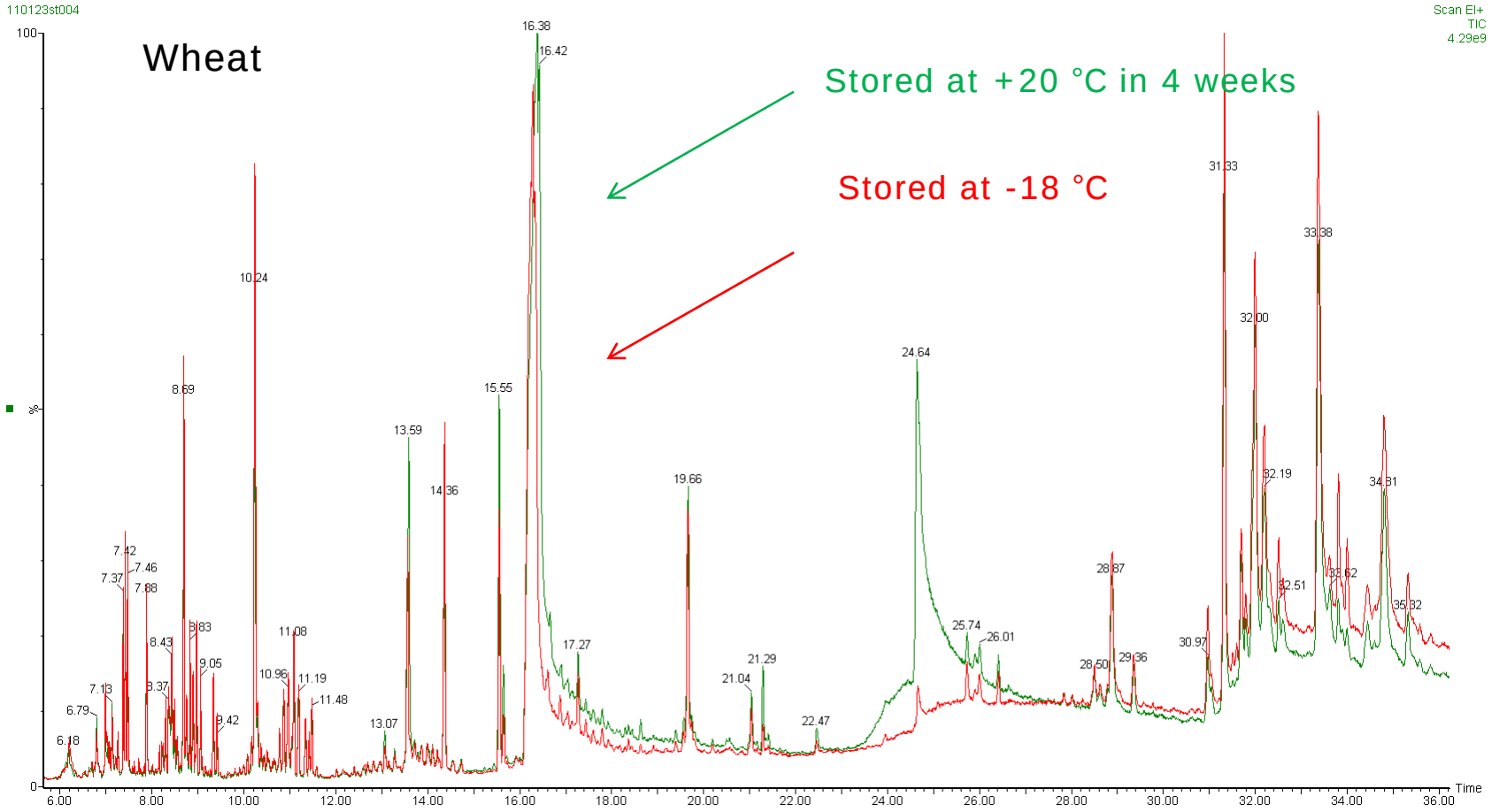
Freeze out step, -80 °C in one hour



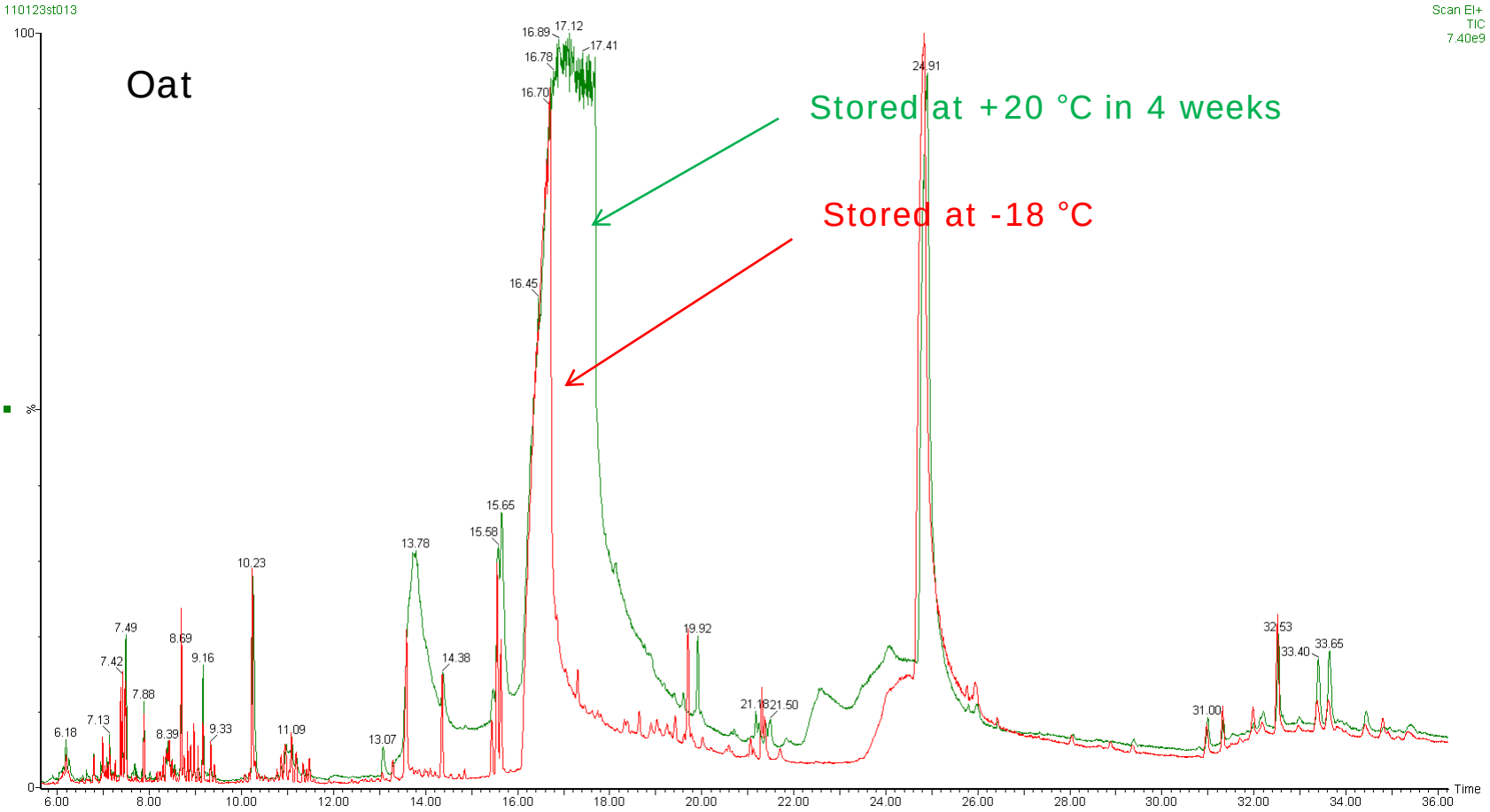
PSA clean-up - wheat



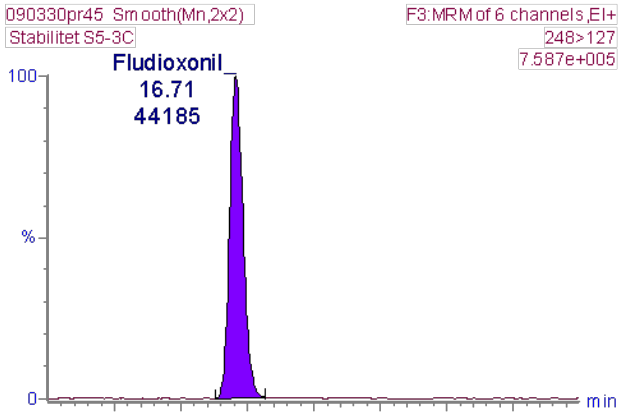
Storage temperature of samples - Freezer or room temperature

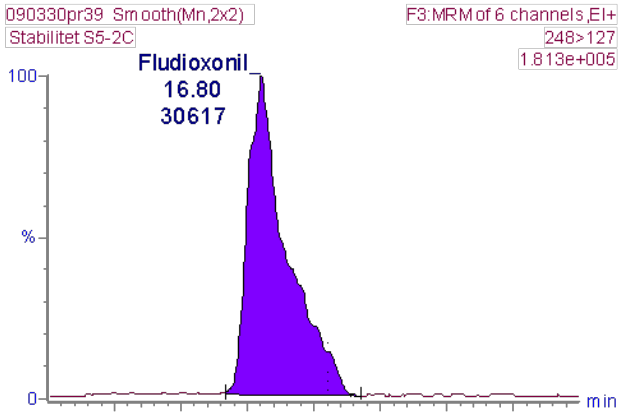


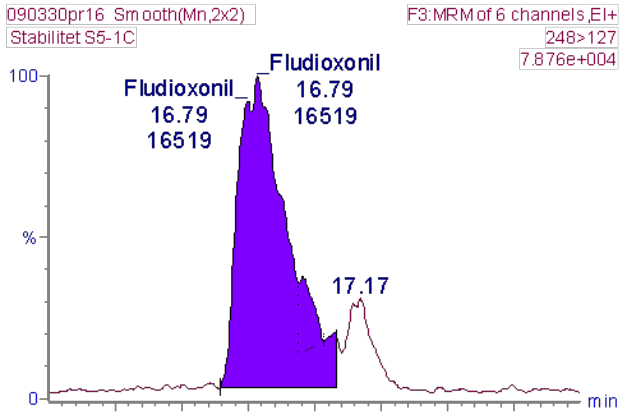
Storage temperature of sample Freezer or roomtemperature

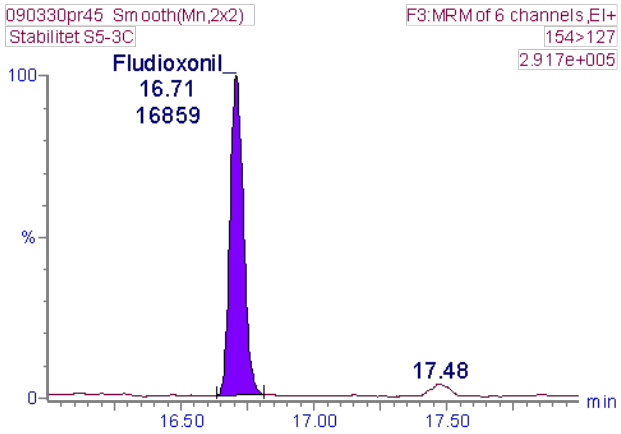


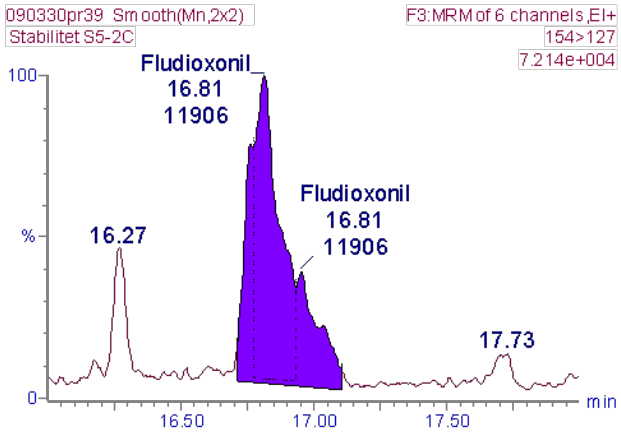
Stability test – EUPT-C3 (oat, Fludioxonil)

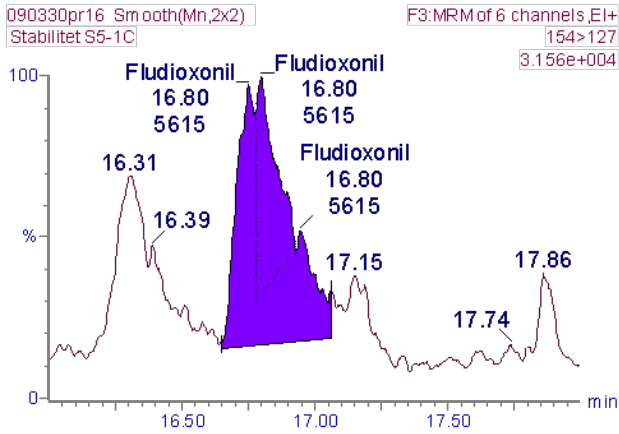












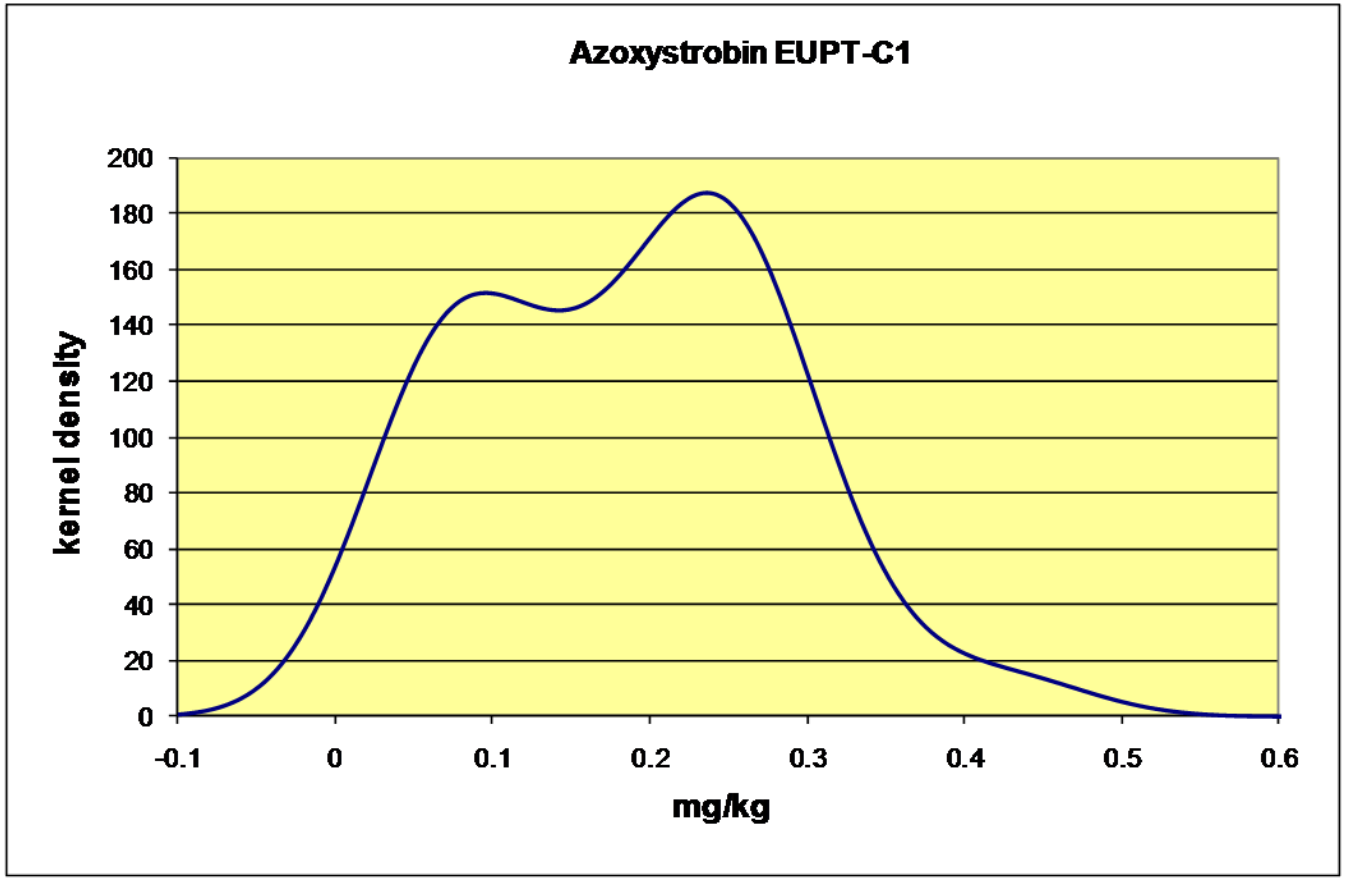
Recommandation

- Cereals are often kept at room temperature because the commodities are not assumed to disintegrate due to the low water content

- However, it is recommended to store laboratory samples in freezer to keep the chemical integrity intact.

- Samples should be stored at -18˚C

Outcome from EUPT-C1, C2



Water addition to sample prior to extraction – EUPT-C2

mg/kg	Median of all results	Median of results with water addition	Median of results with no water addition	Ratio between medians (with / without water)
Alpha-cypermethrin	0.076	0.079	0.072	**1.1**
Bifentrin	0.088	0.087	0.090	**1.0**
Chlorpyrifos-methyl	0.110	0.130	0.056	**2.3**
Iprodione	0.265	0.289	0.100	**2.9**
Malathion *)	0.130	0.168	0.102	**1.6**
Prochloraz *)	0.227	0.239	0.160	**1.5**
Azoxystrobin *)	0.217	0.239	0.133	**1.8**
Trifloxystrobin	0.430	0.439	0.376	**1.2**

***) Spiked in the laboratory**

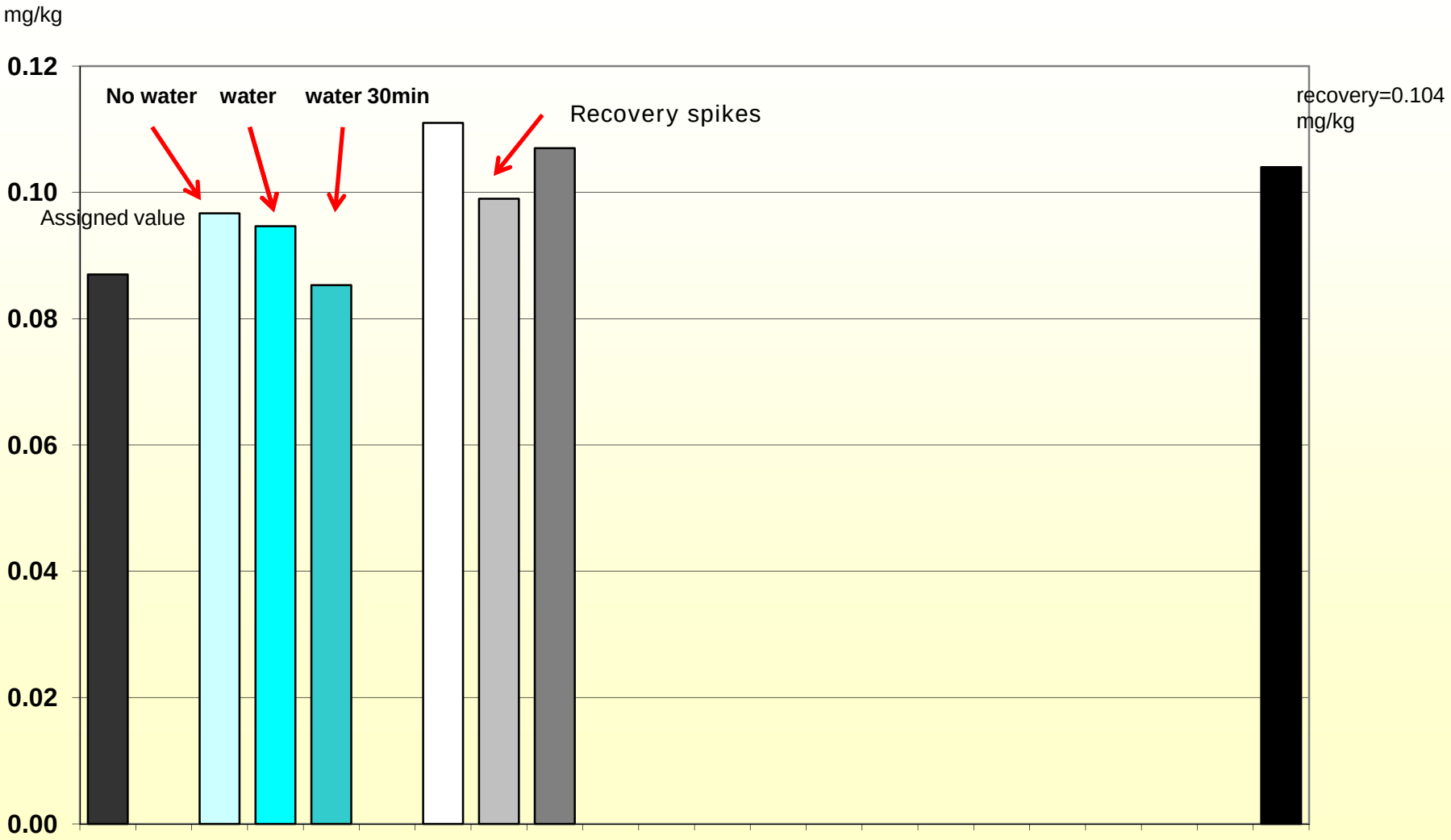
Azoxystrobin PTC1	0.189	0.240	0.074	**3.2**

Experiments on water addition

- QuEChERS method (EN15662)
  - 5 g sample were added 10 g water and then extracted with 10 ml acetonitril for one minute by shaking.
  - A mixture of 4 g magnesium sulphate anhydrous, 1 g Sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate was added the extraction continued for one minute by shaking and centrifuge
  - Six ml of the extract was added 150 mg PSA and 900 mg magnesium sulphate and shaken for 30 seconds.
  - After centrifugation the extract was analysed by GC-MS/MS
- QuEChERS method modified (1)– added water and waited in 30 min before adding acetonitril.
- QuEChERS method modified (2)– not added water

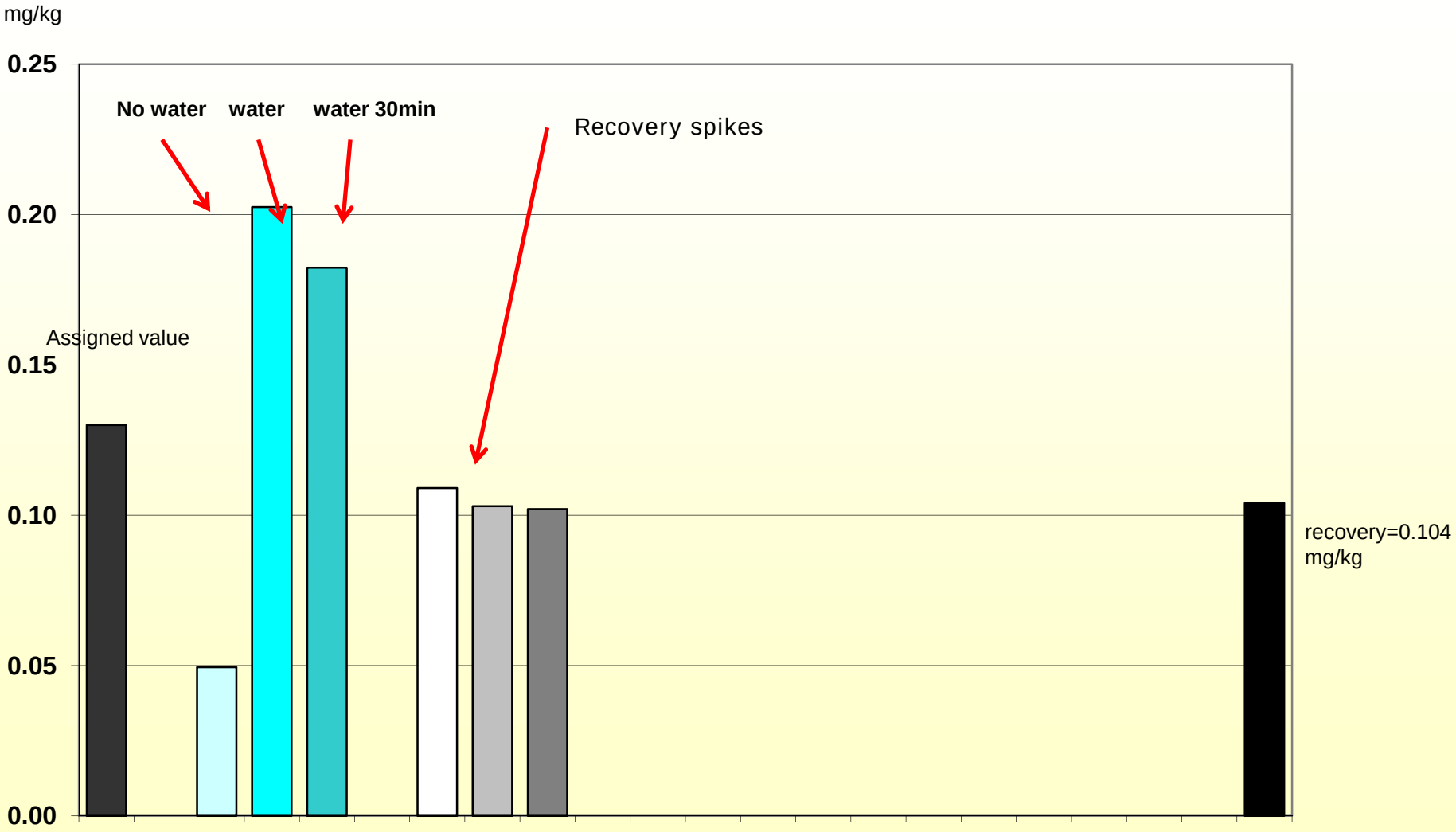
Bifenthrin

PTC2 - incurred



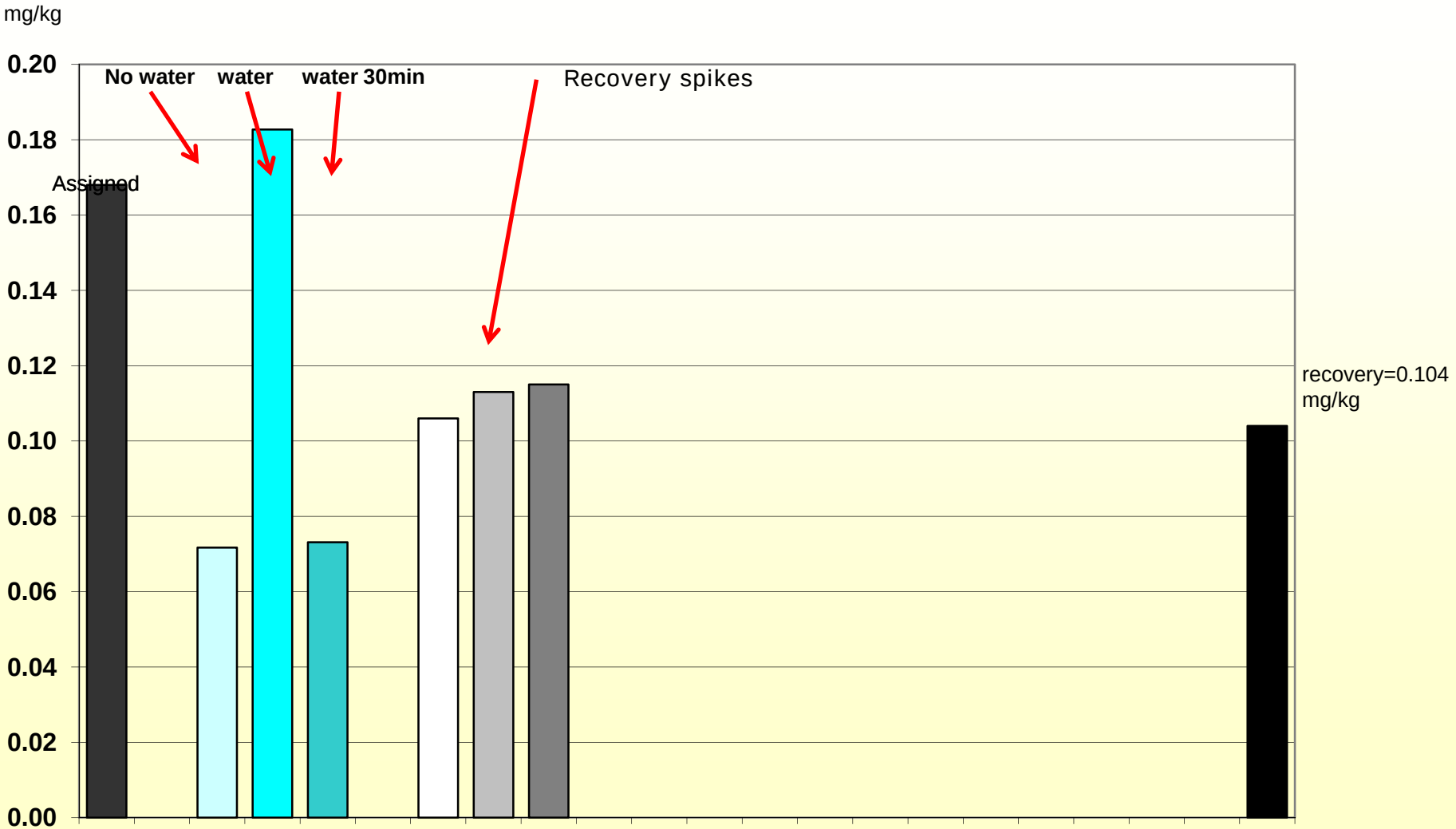
Chlorpyrifos-methyl

PTC2 - incurred



Malathion

PTC2 - spiked



Malation

- 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

– *Hans Mol at CRL/NRL workshop in Copenhagen 2009*

Recommandations in Document No. SANCO/10684/2009

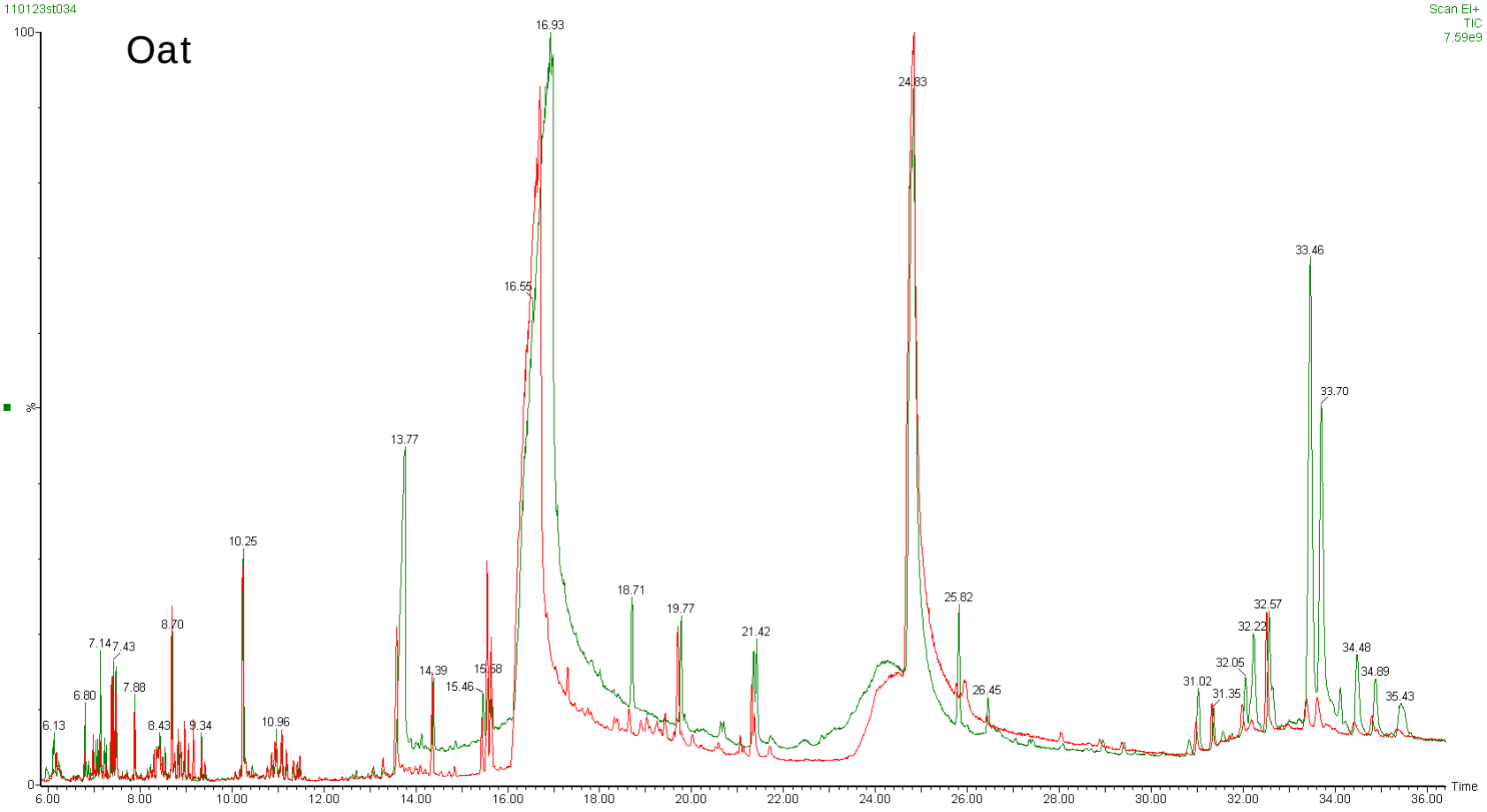
- METHOD VALIDATION AND QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED

  - To improve the extraction efficiency of low moisture containing commodities (cereals, dried fruits), it is recommended to add water to the samples before extraction is carried out.
  - However, the time between addition of water and extraction should be controlled in order to avoid any significant losses of pesticides

Experiments with different solvent

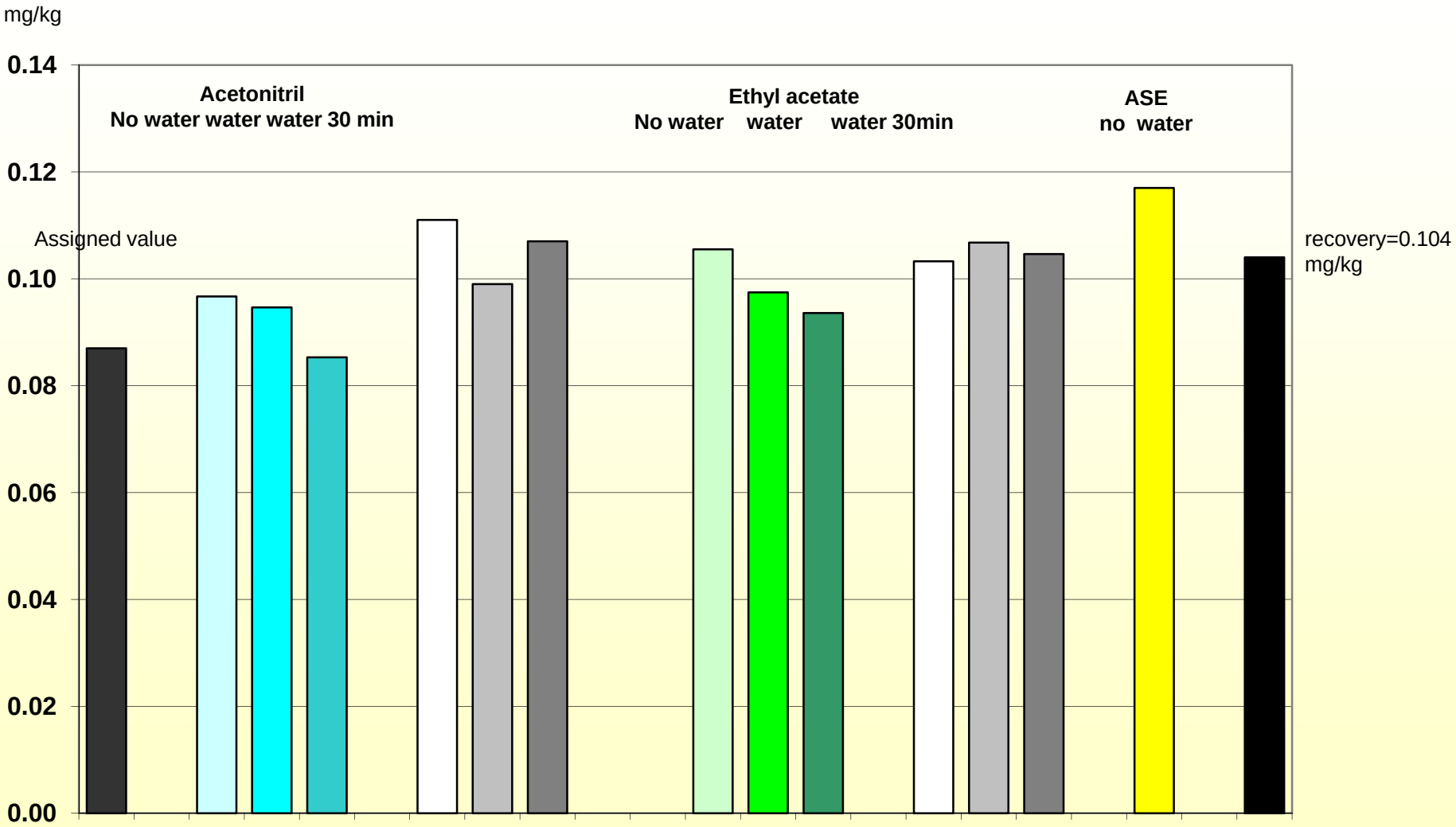
- QuEChERS method (EN15662)
  - 5 g sample were added 10 g water and then extracted with 10 ml acetonitril for one minute by shaking etc.
- QuEChERS method modified (3)– acetonitil change to ethyl acetate.
- ASE method
  - 5 g samples into extraction cells and filled to the top with sand.
  - The oven temperature was set at 70 °C and cell pressure at 1500 psi.
  - Acetonitril was introduced and heated to the set temperature in 5 min, followed by another 3 min of static time of extraction.
  - The extracts were evaporated to 10 ml.
  - The clean up procedure followed the QuEChERS method.

Acetonitril and ethyl acetate



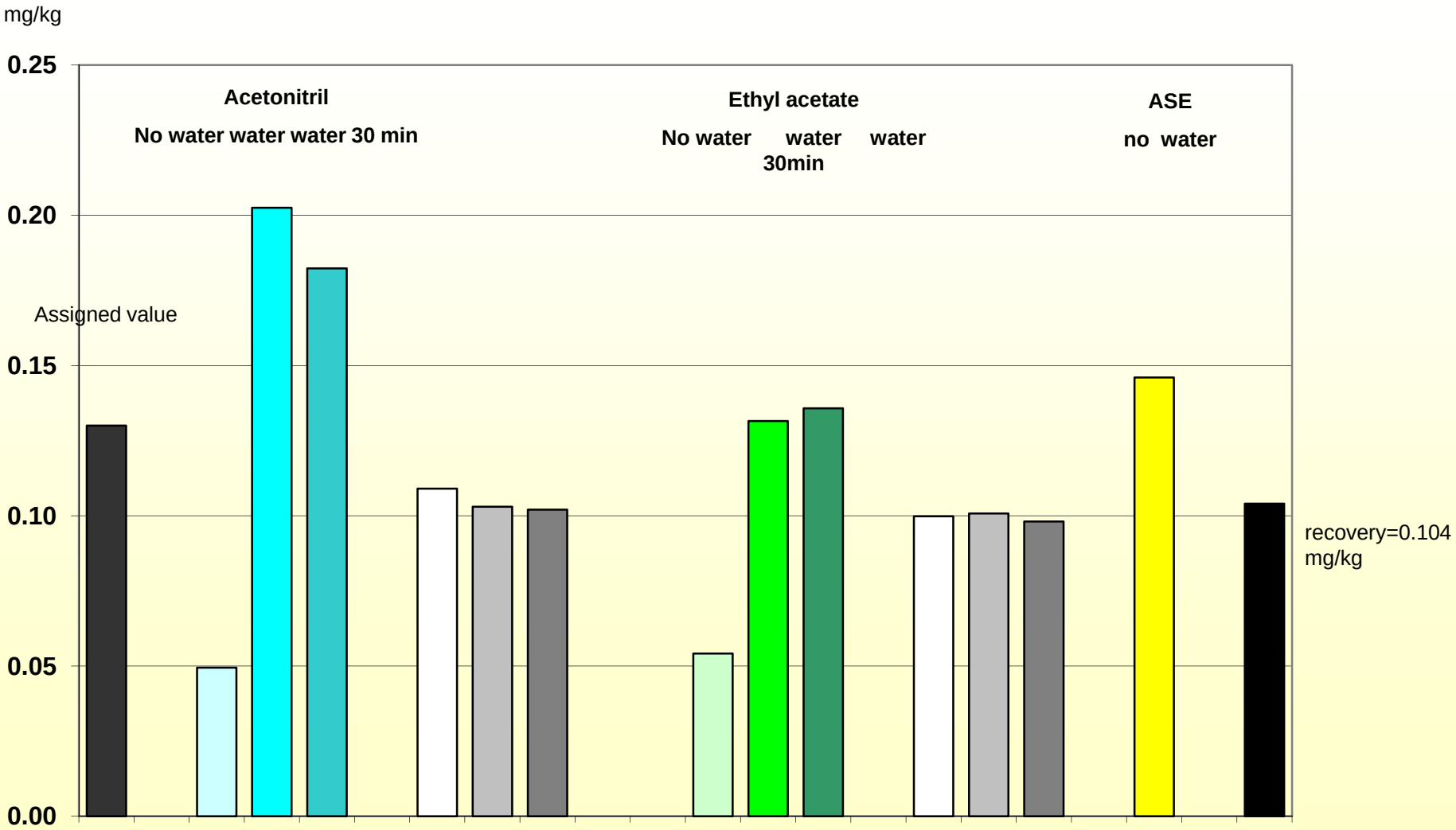
Bifenthrin

PTC2 - incurred



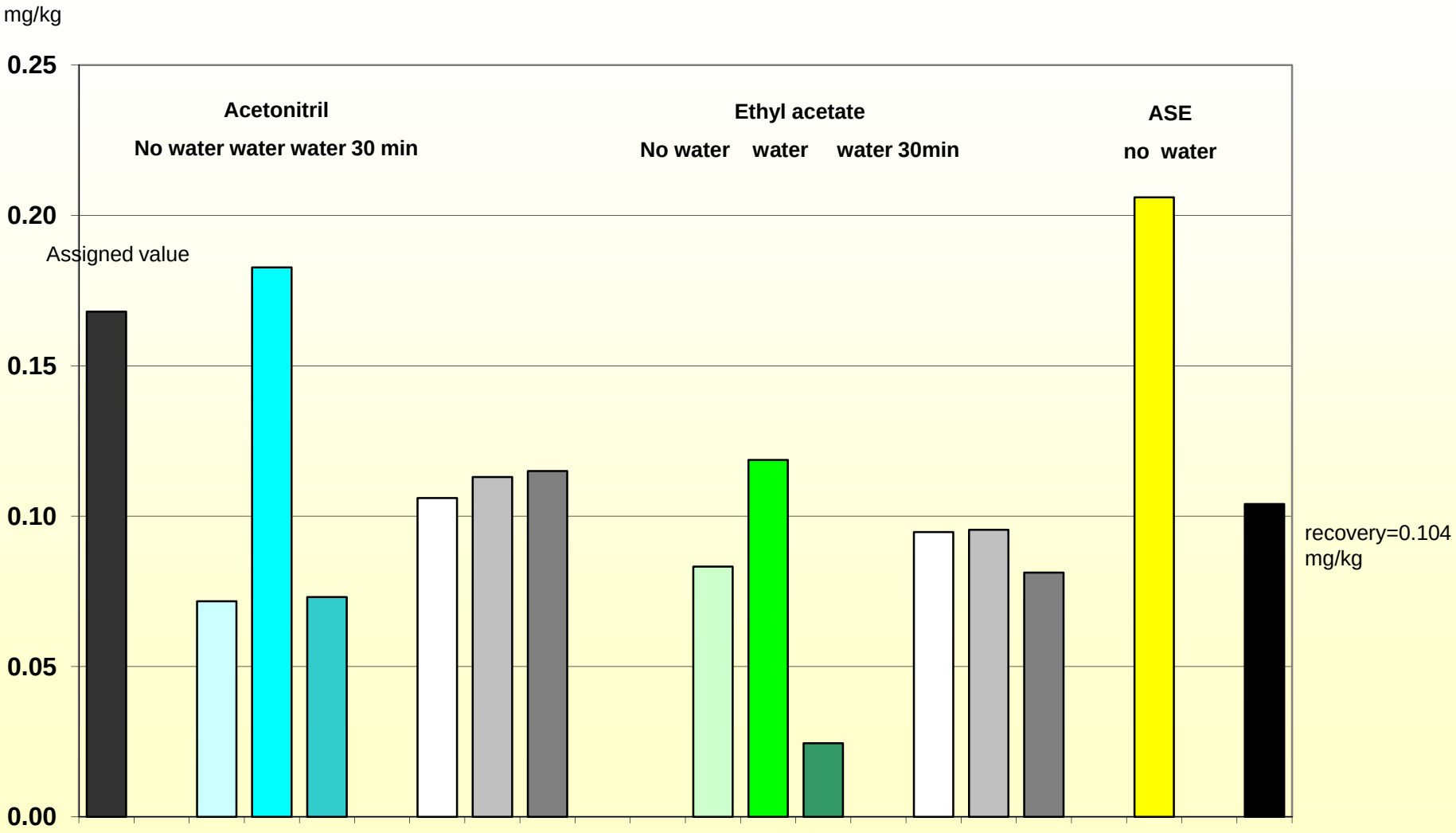
Chlorpyrifos-methyl

PTC2 - incurred



Malathion

PTC2 - incurred



Results from Proficiency test EUPT-C4

- Multi variate statistical methods
- Procedure

  - Robustify by replacing observations by
    - Ranks or
    - Normal scores – done here

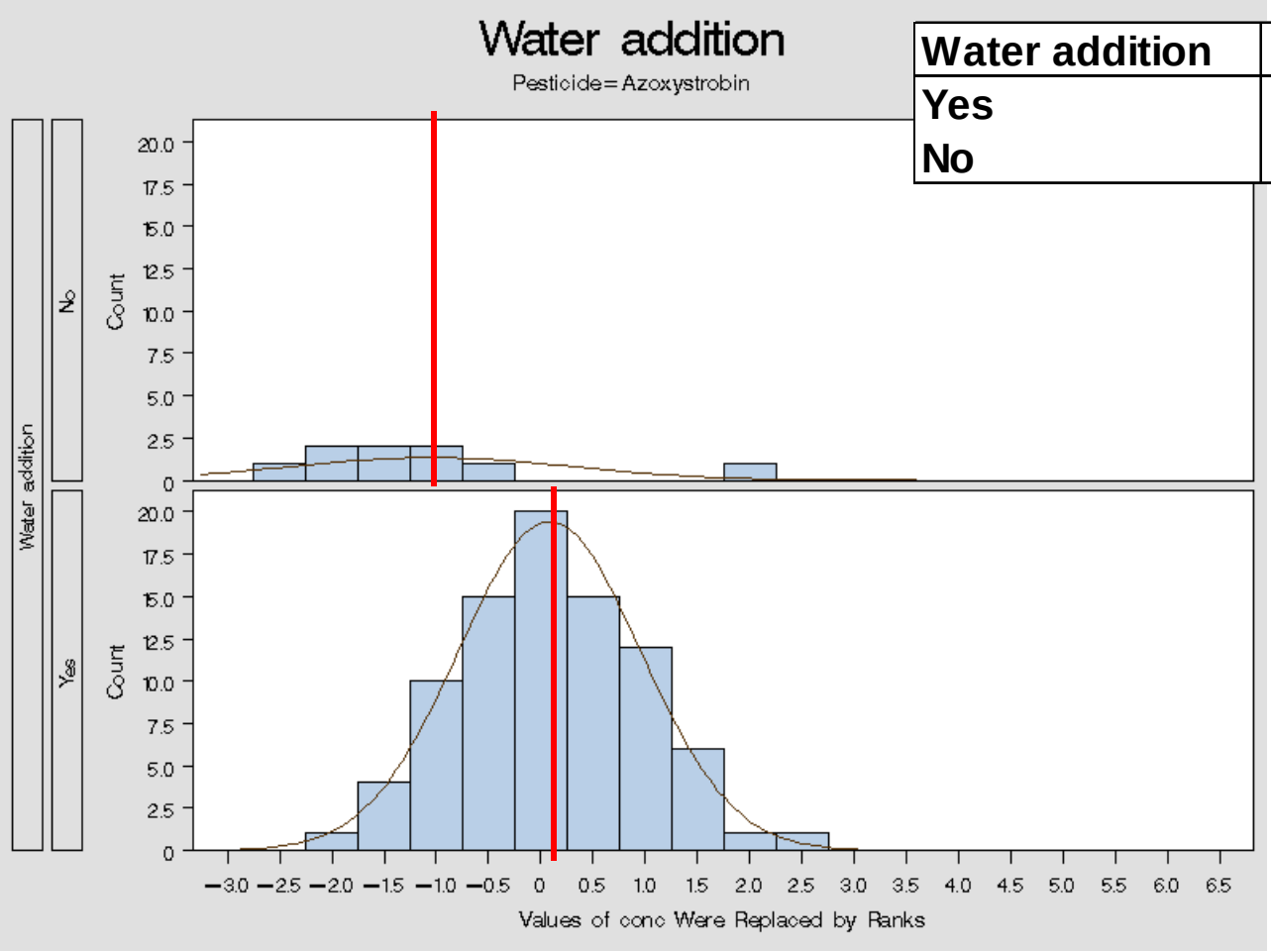
  - Analyse by means of ANOVA/ANCOVA
    - Variable vs. factor(s) (change in mean)
    - abs(variable-mean) vs. factor(s) (change in “variance”)

  - Analyse by means of factor analysis

Pesticides - concentration – by Water Addition

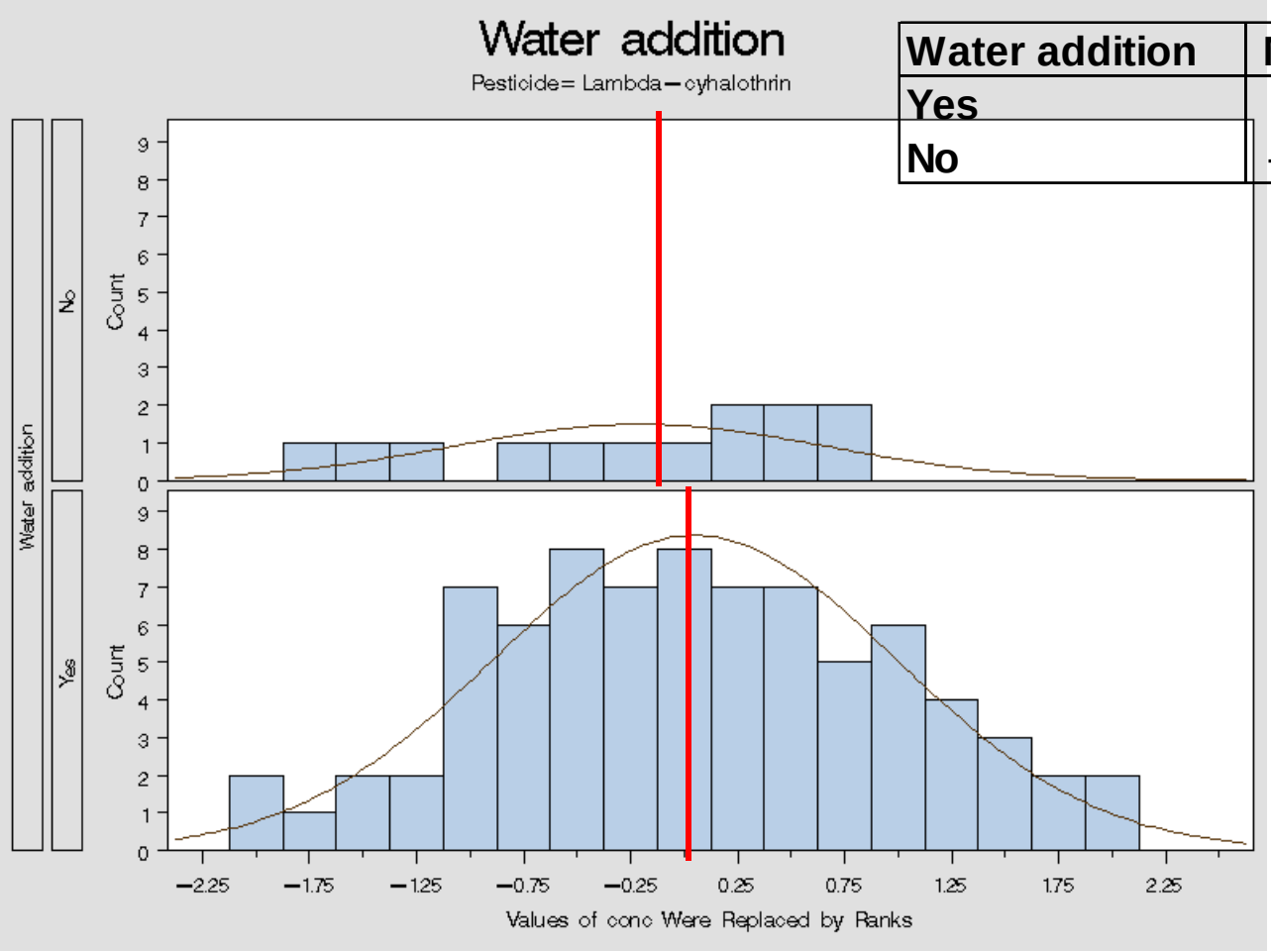
- Differences between water added yes/no

Azoxystrobin



Water addition	Mean	N	Group
Yes	0.08	85	A
No	-1.05	9	B

Lambda-cyholothrin



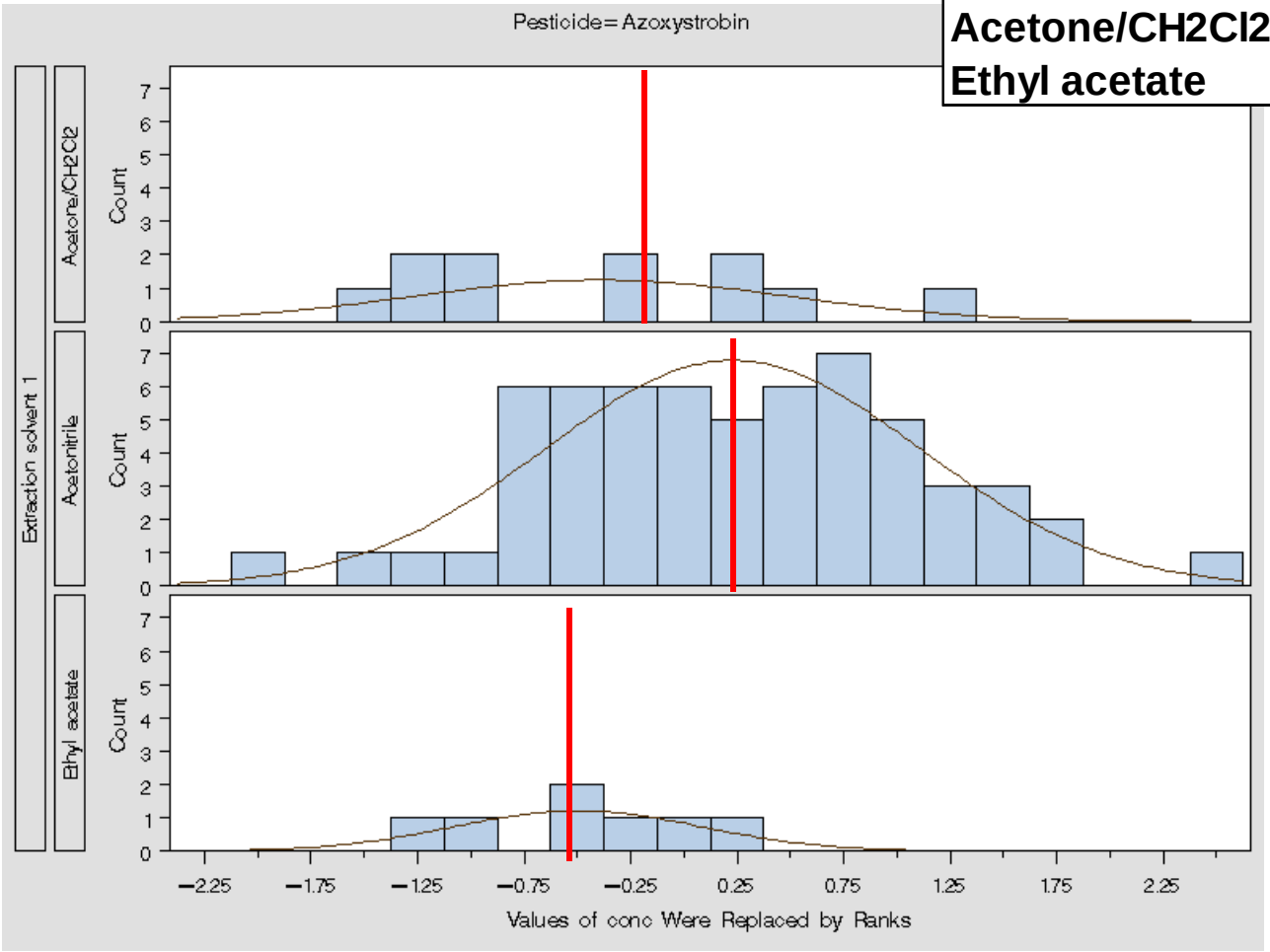
Water addition	Mean	N	Group
Yes	0.05	80	A
No	-0.21	13	A

Pesticides - concentration – by Extraction Solvent 1

- Differences between extraction solvents
  - Acetonitril
  - Acetone/CH2Cl2
  - Ethyl acetate

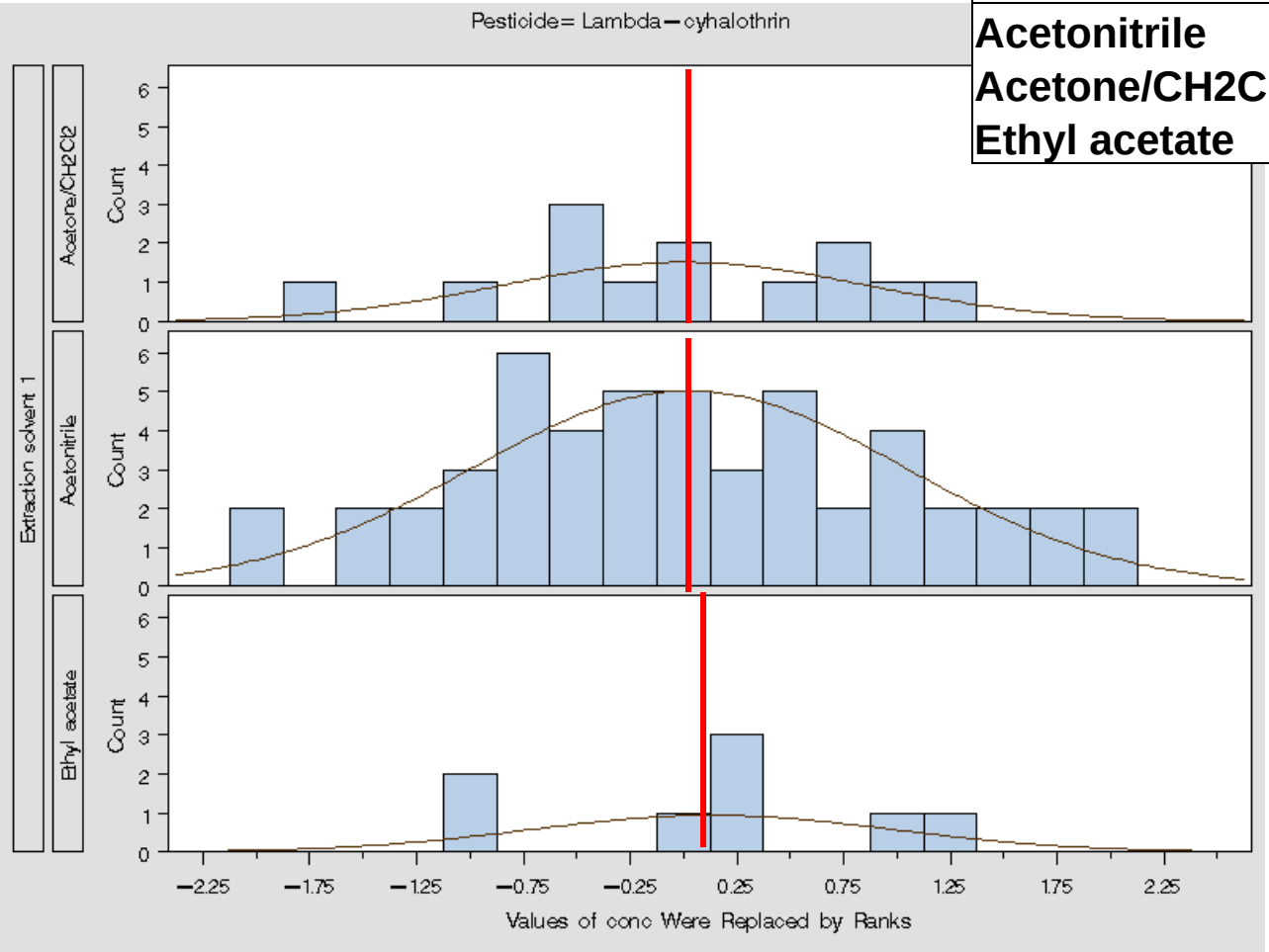
Azoxystrobin

Solvent	Mean	N	Group
Acetonitrile	0.23	60	A
Acetone/CH2Cl2	-0.39	11	AB
Ethyl acetate	-0.50	7	B



Lambda-cyholothrin

Solvent	Mean	N	Group
Acetonitrile	0.03	60	A
Acetone/CH2Cl2	0.01	11	A
Ethyl acetate	0.13	7	A



Two sided analysis of variance

- Factor 1: solvent

Acetone	7
Acetone/CH2Cl2	26
Acetone/ea	3
Acetonitrile	66
Cyclohexane	1
Dichloromethane	1
Ethyl acetate	12
Methanol	6

- Factor 2: water addition

Yes	89
No	15

Water addition and Extraction solvent 1

Pesticide	Mean shift			Variance shift		
p values	Water	Solv	W*S	Water	Solv	W*S
Azoxystrobin	0.006	0.63	0.03	<0.001	0.09	0.38
Carbaryl	0.15	0.72	0.35	0.30	0.61	0.48
Carbendazim&benomyl	0.003	0.14	0.74	0.15	0.38	0.40
Chlorpyrifos-methyl	0.22	0.53	0.43	0.76	0.82	0.93
Deltametrin (cis)	0.29	0.28	0.81	0.52	0.18	0.53
Fenitrothion	0.68	0.75	0.60	0.99	0.65	0.62
Fenpropimorph	0.008	0.10	0.90	0.76	0.92	0.16
Fluquinconazole	0.053	0.72	0.13	0.18	0.09	0.25
Flutriafol	0.008	0.86	0.50	0.02	0.56	0.43
Isoproturon	0.42	0.18	0.001	0.002	0.74	0.79
Kresoxim-methyl	0.002	0.34	0.03	0.01	0.11	0.15
Lambda-cyhalothrin	0.41	0.54	0.17	0.33	0.31	0.13
- Malation	0.02	0.47	0.64	0.38	0.47	0.64
Pirimiphos-methyl	0.07	0.43	0.16	0.76	0.80	0.62
Spiroxamine	0.20	0.64	0.55	0.03	0.84	0.54
- Triadimenol	0.18	0.26	0.83	0.49	0.15	0.13

Factor analysis
Rotated Factor Pattern

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Malathion	0.876	0.299	-0.022	0.134	0.060	organophosphate
Kresoxim-methyl	0.861	0.024	-0.139	0.141	0.224	strobilurin
Azoxystrobin	0.849	-0.009	0.032	-0.125	-0.148	strobilurin
Pirimiphos-methyl	0.809	0.307	0.085	0.082	0.100	organophosphate
Fenitrothion	0.793	0.126	-0.139	0.084	-0.114	organophosphate
Chlorpyrifos-methyl	0.703	0.458	-0.005	0.057	-0.025	organophosphate
Triadimenol	0.695	0.098	0.026	0.109	0.289	triazole
Spiroxamine	0.635	-0.082	0.354	-0.169	-0.407	
Deltamethrin (cis)	0.212	0.826	-0.082	0.008	-0.027	pyrethroid
Lambda-cyhalothrin	0.187	0.708	-0.270	-0.034	0.015	pyrethroid
Isoproturon	0.232	-0.274	0.788	-0.144	0.184	urea
Carbendazim and benom	-0.312	-0.140	0.717	0.099	0.053	benzimidazole
Fenpropimorph	0.023	-0.371	-0.146	0.798	-0.033	morpholine
Fluquinconazole	0.178	0.335	0.090	0.646	0.223	triazole
Flutriafol	0.078	0.116	0.522	0.544	-0.180	triazole
Carbaryl	0.085	-0.025	0.127	0.012	0.943	carbamate

Conclusions

- Co-extracted compounds
  - Clean-up with a freezing out step and/or PSA did only remove some of the co-extracted compounds
- The extraction efficiency of incurred pesticides
  - was significantly increased when water was added to the samples before extraction.
    - Especially for phosphor pesticides, azole pesticides, and strobilurins
  - was not increased if the samples were left for 30 min after water addition.
    - For some pesticides (malathion) the extraction efficiency droped, due to degradation
- Statictical analysis of EUPT-C4 results
  - Showed significant shift in mean values between adding water or not
  - showed a minor shift in the mean values between acetonitril and ethylactate


Thank you for your attention
Gracias por su atención