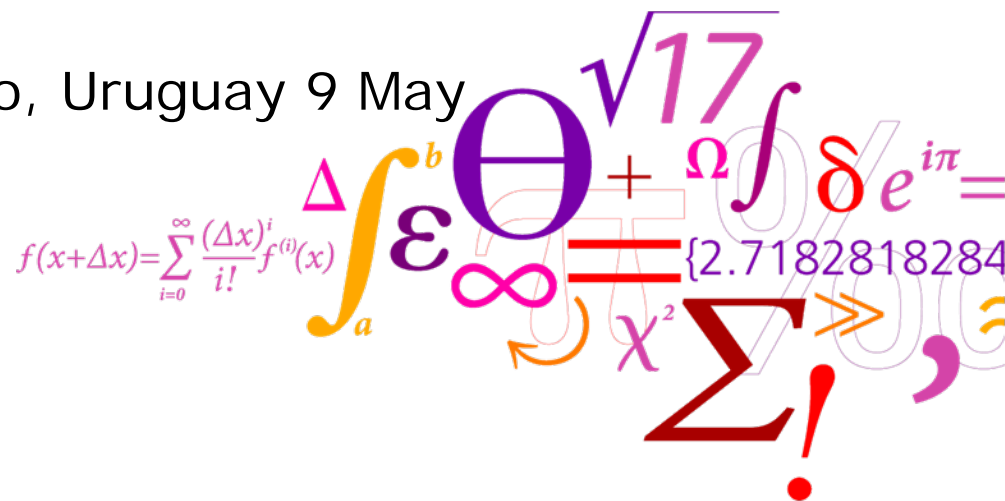


# Co-extracted compounds from cereals and parameters affecting the extraction and clean-up efficiency of incurred pesticide residues

Mette Erecius Poulsen, Bjarne Kjær Ersbøll,  
Susan Strange Herrmann and Hanne Bjerre  
Christensen

LAPRW2011 – Montevideo, Uruguay 9 May









# 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

## 27 Member States

### Joined in 1957

	<b>Belgium</b> 10.5 million 24 MEPs
	<b>France</b> 62.8 million 78 MEPs
	<b>Germany</b> 82.4 million 99 MEPs
	<b>Italy</b> 58.7 million 78 MEPs
	<b>Luxembourg</b> 0.4 million 6 MEPs
	<b>Netherlands</b> 16.3 million 27 MEPs

### Joined in 1973

	<b>Denmark</b> 5.4 million 14 MEPs
	<b>Ireland</b> 4.2 million 13 MEPs
	<b>United Kingdom</b> 60.3 million 78 MEPs




### Joined in 1981

	<b>Greece</b> 11.1 million 24 MEPs
--	--

### Joined in 1986

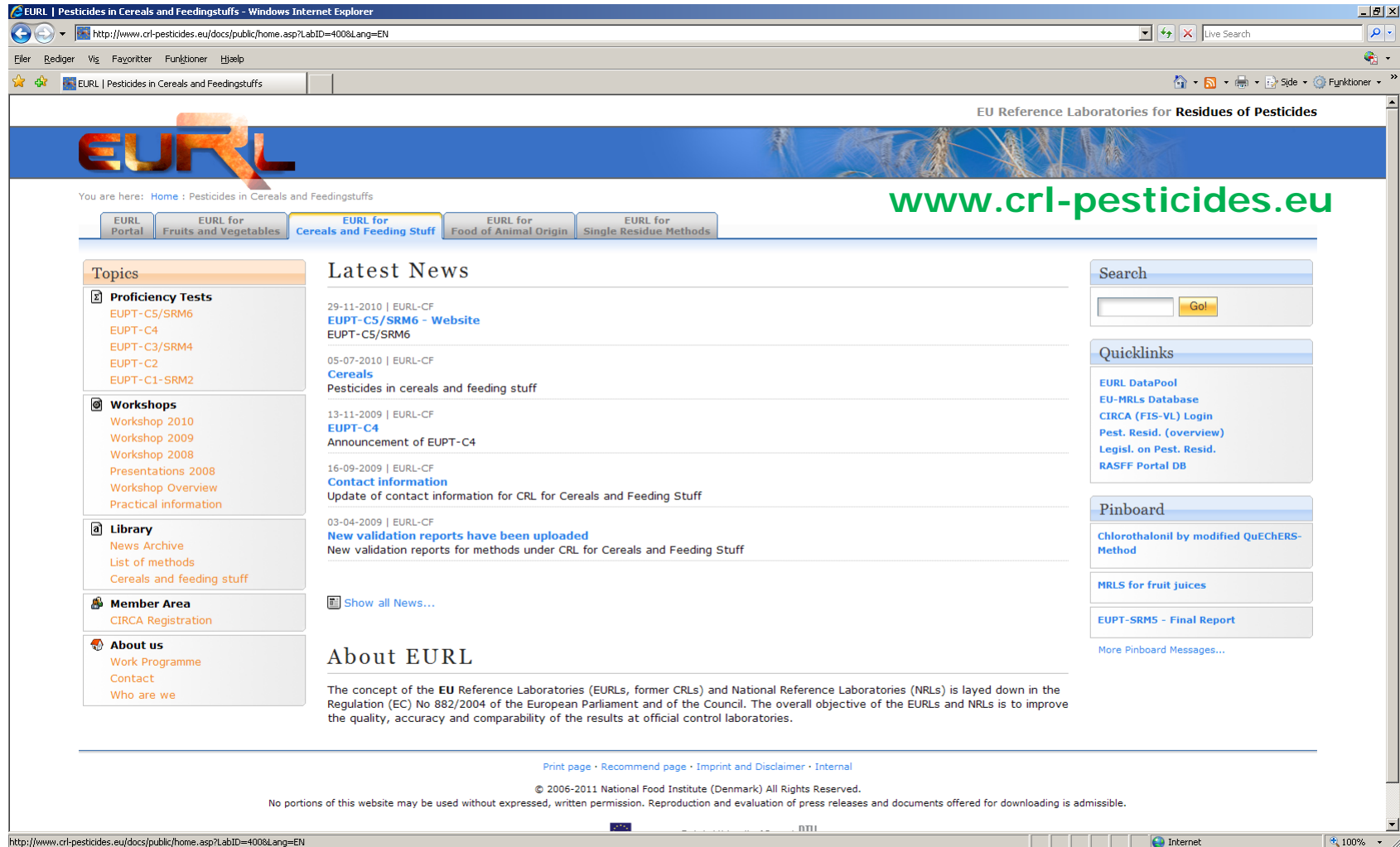
	<b>Portugal</b> 10.5 million 24 MEPs
	<b>Spain</b> 43.7 million 54 MEPs

### Joined in 1995

	<b>Austria</b> 8.2 million 18 MEPs
	<b>Finland</b> 5.2 million 14 MEPs
	<b>Sweden</b> 9.0 million 19 MEPs



# EU reference laboratories for pesticide residues - web portal

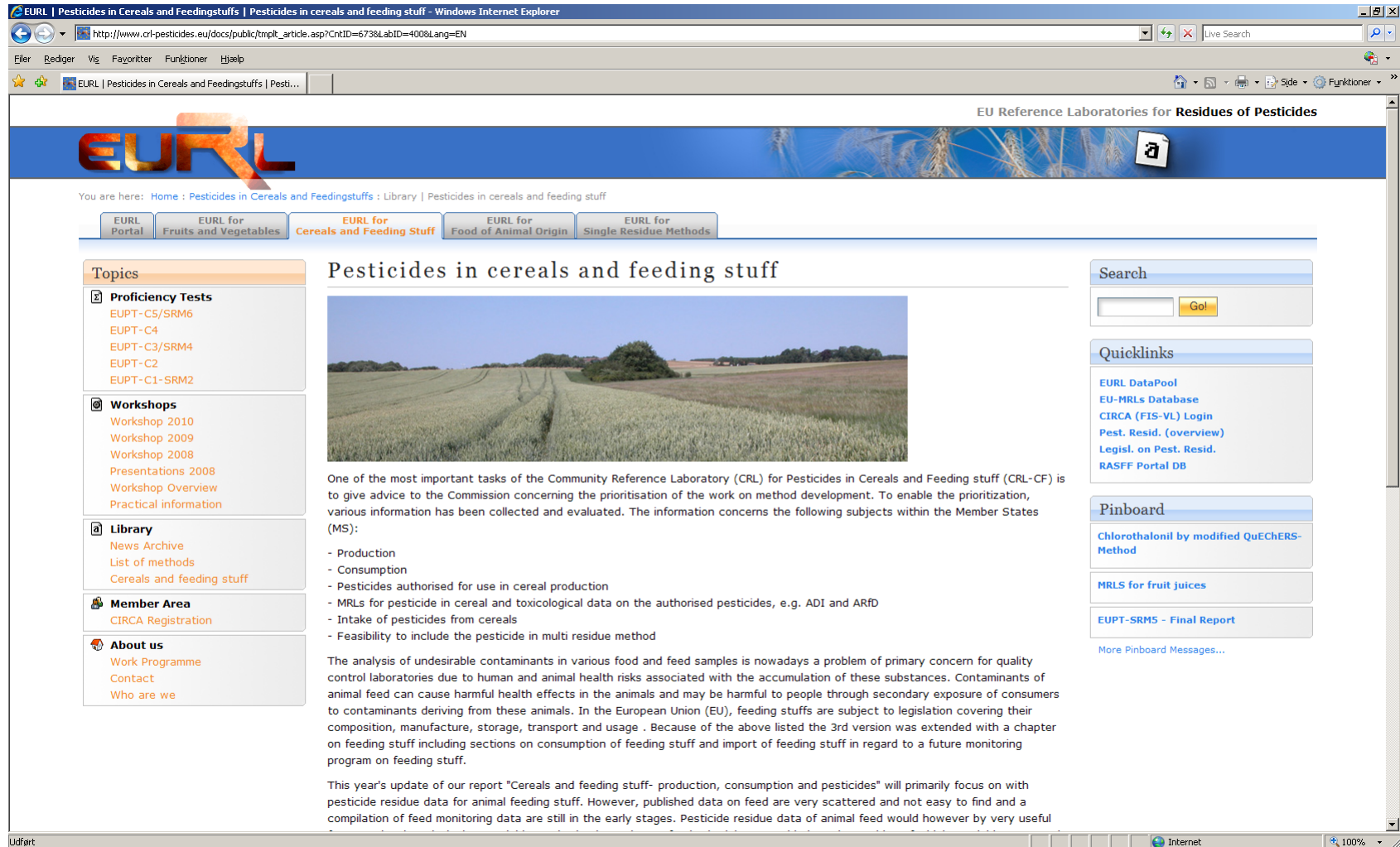


The screenshot shows the EURL website for Pesticides in Cereals and Feedingstuffs. The browser window is titled "EURL | Pesticides in Cereals and Feedingstuffs - Windows Internet Explorer". The address bar shows the URL: <http://www.crl-pesticides.eu/docs/public/home.asp?LabID=400&Lang=EN>. The website header features the EURL logo and the text "EU Reference Laboratories for Residues of Pesticides". Below the header, there is a navigation bar with tabs for "EURL Portal", "EURL for Fruits and Vegetables", "EURL for Cereals and Feeding Stuff" (selected), "EURL for Food of Animal Origin", and "EURL for Single Residue Methods". The main content area is divided into several sections:

- Topics:** A sidebar menu with links to Proficiency Tests (EUPT-C5/SRM6, EUPT-C4, EUPT-C3/SRM4, EUPT-C2, EUPT-C1-SRM2), Workshops (Workshop 2010, Workshop 2009, Workshop 2008, Presentations 2008, Workshop Overview, Practical information), Library (News Archive, List of methods, Cereals and feeding stuff), Member Area (CIRCA Registration), and About us (Work Programme, Contact, Who are we).
- Latest News:** A section with three news items:
  - 29-11-2010 | EURL-CF: [EUPT-C5/SRM6 - Website](#) (EUPT-C5/SRM6)
  - 05-07-2010 | EURL-CF: [Cereals](#) (Pesticides in cereals and feeding stuff)
  - 13-11-2009 | EURL-CF: [EUPT-C4](#) (Announcement of EUPT-C4)
  - 16-09-2009 | EURL-CF: [Contact information](#) (Update of contact information for CRL for Cereals and Feeding Stuff)
  - 03-04-2009 | EURL-CF: [New validation reports have been uploaded](#) (New validation reports for methods under CRL for Cereals and Feeding Stuff)
- Search:** A search bar with a "Go!" button.
- Quicklinks:** A list of links including [EURL DataPool](#), [EU-MRLs Database](#), [CIRCA \(FIS-VL\) Login](#), [Pest. Resid. \(overview\)](#), [Legisl. on Pest. Resid.](#), and [RASFF Portal DB](#).
- Pinboard:** A section with links to [Chlorothalonil by modified QuEChERS-Method](#), [MRLs for fruit juices](#), and [EUPT-SRM5 - Final Report](#).
- About EURL:** A section explaining the concept of the EU Reference Laboratories (EURLs, former CRLs) and National Reference Laboratories (NRLs) under Regulation (EC) No 882/2004. The objective is to improve the quality, accuracy, and comparability of the results at official control laboratories.

At the bottom of the page, there is a footer with links for "Print page", "Recommend page", "Imprint and Disclaimer", and "Internal". Below these links is a copyright notice: "© 2006-2011 National Food Institute (Denmark) All Rights Reserved." and a statement: "No portions of this website may be used without expressed, written permission. Reproduction and evaluation of press releases and documents offered for downloading is admissible."

# EU reference laboratories for pesticide residues - web portal



The screenshot shows the EURL website for Pesticides in Cereals and Feedingstuffs. The browser window is titled "EURL | Pesticides in Cereals and Feedingstuffs | Pesticides in cereals and feeding stuff - Windows Internet Explorer". The address bar shows the URL: <http://www.crl-pesticides.eu/docs/public/tmpl/article.asp?CrtID=673&LabID=400&Lang=EN>. The website header features the EURL logo and the text "EU Reference Laboratories for Residues of Pesticides". Below the header, a navigation bar includes links for "EURL Portal", "EURL for Fruits and Vegetables", "EURL for Cereals and Feeding Stuff", "EURL for Food of Animal Origin", and "EURL for Single Residue Methods". The main content area is titled "Pesticides in cereals and feeding stuff" and features a large image of a field. The text below the image states: "One of the most important tasks of the Community Reference Laboratory (CRL) for Pesticides in Cereals and Feeding stuff (CRL-CF) is to give advice to the Commission concerning the prioritisation of the work on method development. To enable the prioritization, various information has been collected and evaluated. The information concerns the following subjects within the Member States (MS):" followed by a list of topics: Production, Consumption, Pesticides authorised for use in cereal production, MRLs for pesticide in cereal and toxicological data on the authorised pesticides, e.g. ADI and ARfD, Intake of pesticides from cereals, and Feasibility to include the pesticide in multi residue method. The text continues: "The analysis of undesirable contaminants in various food and feed samples is nowadays a problem of primary concern for quality control laboratories due to human and animal health risks associated with the accumulation of these substances. Contaminants of animal feed can cause harmful health effects in the animals and may be harmful to people through secondary exposure of consumers to contaminants deriving from these animals. In the European Union (EU), feeding stuffs are subject to legislation covering their composition, manufacture, storage, transport and usage. Because of the above listed the 3rd version was extended with a chapter on feeding stuff including sections on consumption of feeding stuff and import of feeding stuff in regard to a future monitoring program on feeding stuff. This year's update of our report "Cereals and feeding stuff- production, consumption and pesticides" will primarily focus on with pesticide residue data for animal feeding stuff. However, published data on feed are very scattered and not easy to find and a compilation of feed monitoring data are still in the early stages. Pesticide residue data of animal feed would however by very useful". The left sidebar contains sections for "Topics" (Proficiency Tests, Workshops, Library, Member Area, About us) and "Search". The right sidebar contains sections for "Quicklinks" (EURL DataPool, EU-MRLs Database, CIRCA (FIS-VL) Login, Pest. Resid. (overview), Legis. on Pest. Resid., RASFF Portal DB) and "Pinboard" (Chlorothalonil by modified QuEChERS-Method, MRLs for fruit juices, EUPIT-SRM5 - Final Report).

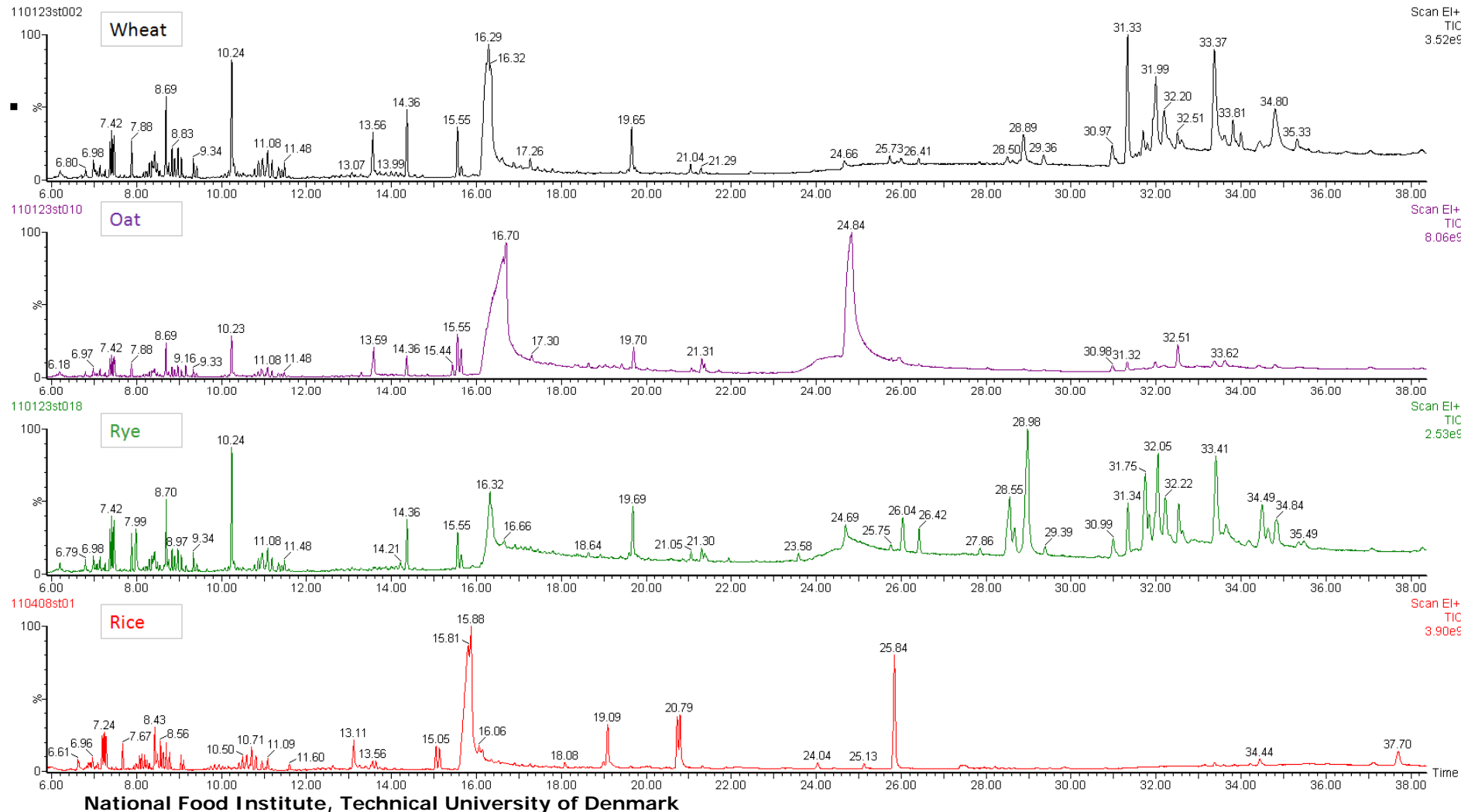
# EUPTs / cereals – overview

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

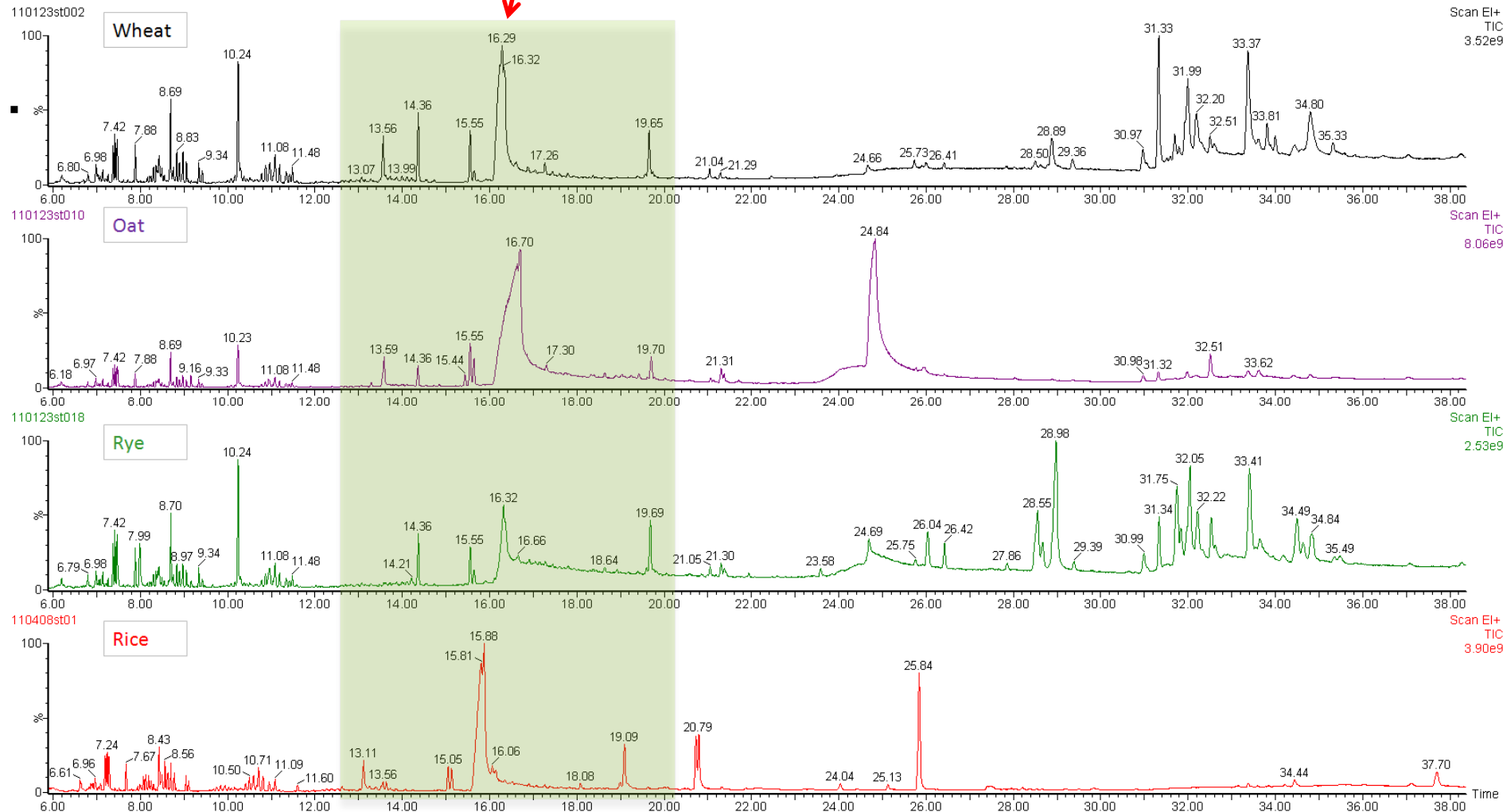


# Coextracted compounds from cereals

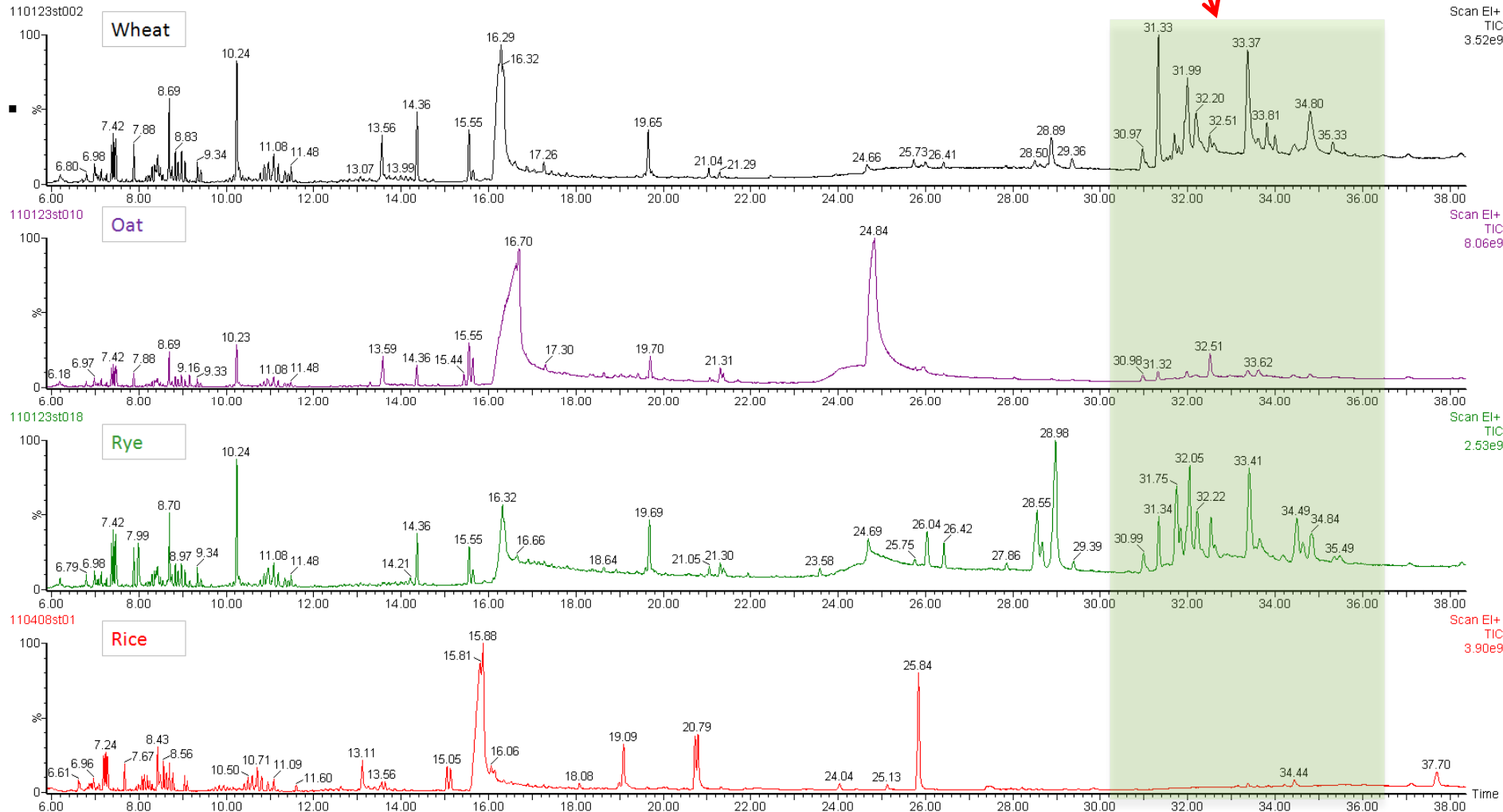
## Quechers method – GC/MS scan mode



Fatty acids



# Phytosterols

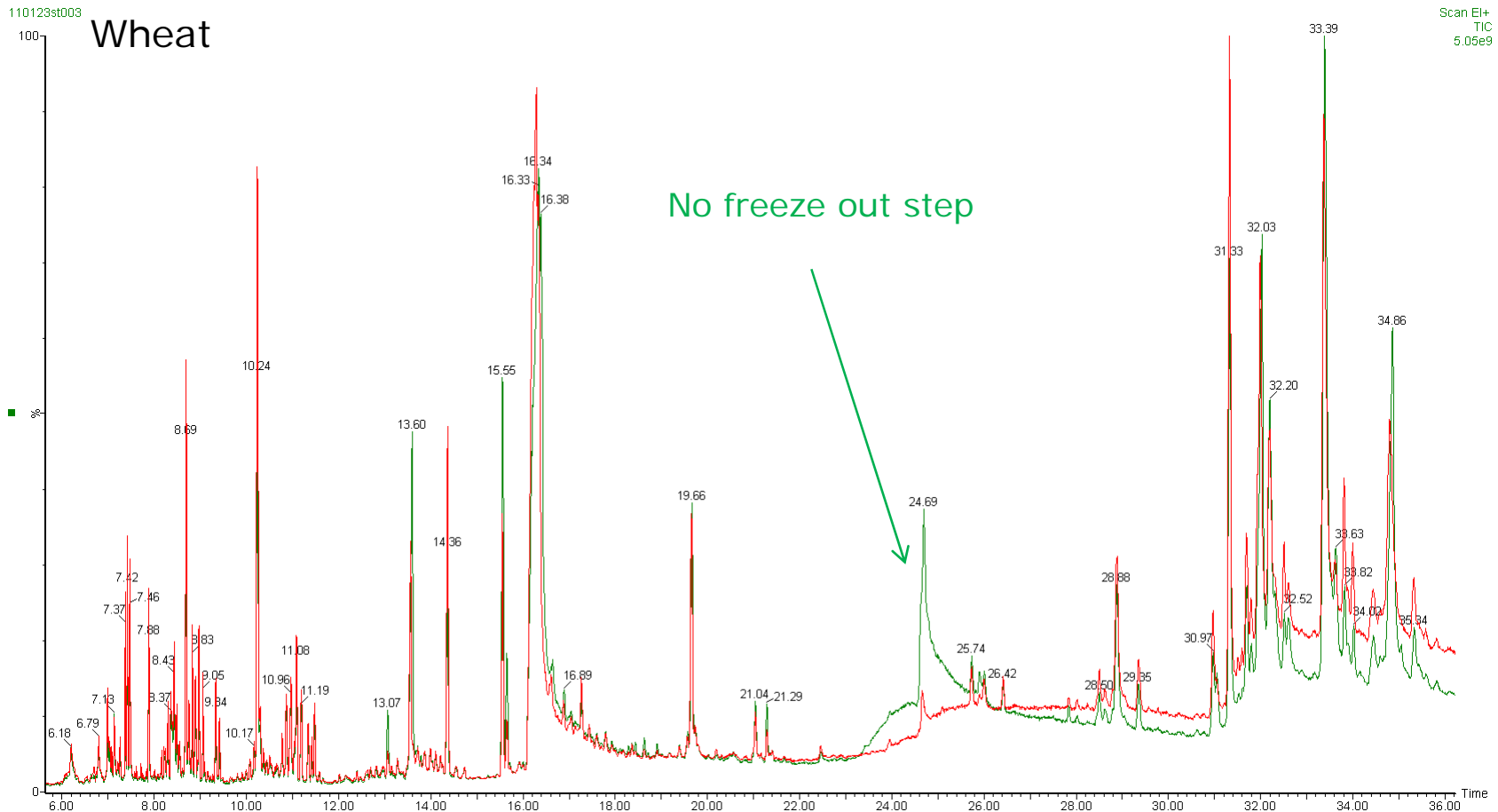


## Freeze out step, -80 °C in one hour

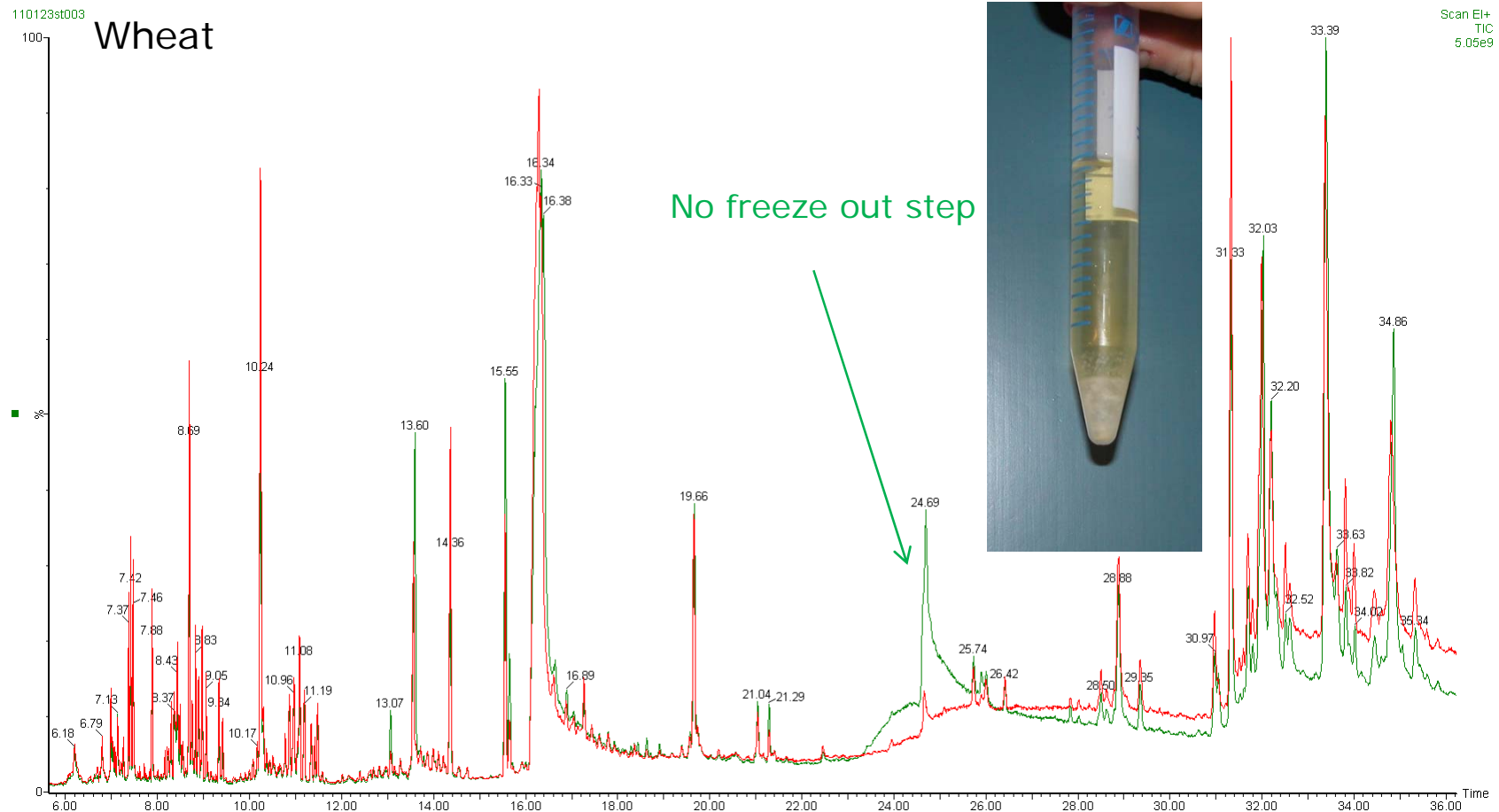
- 8 ml of raw extract of Quechers method are transferred to a 15 ml tube and put in a -80 °C freezer for one hour.
- Centrifugation at in cold centrifuge for 5 min



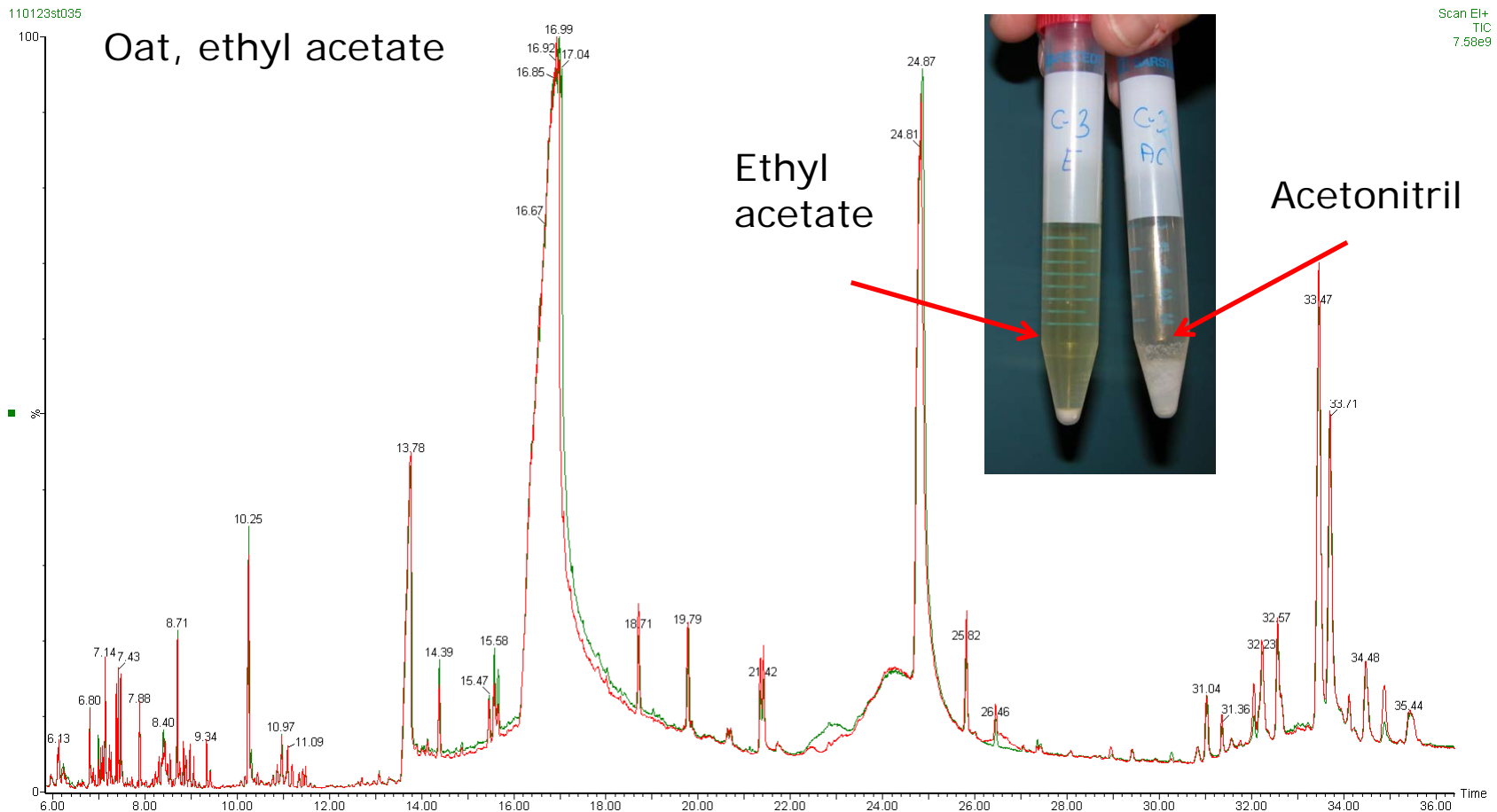
# Freeze out step, -80 °C in one hour



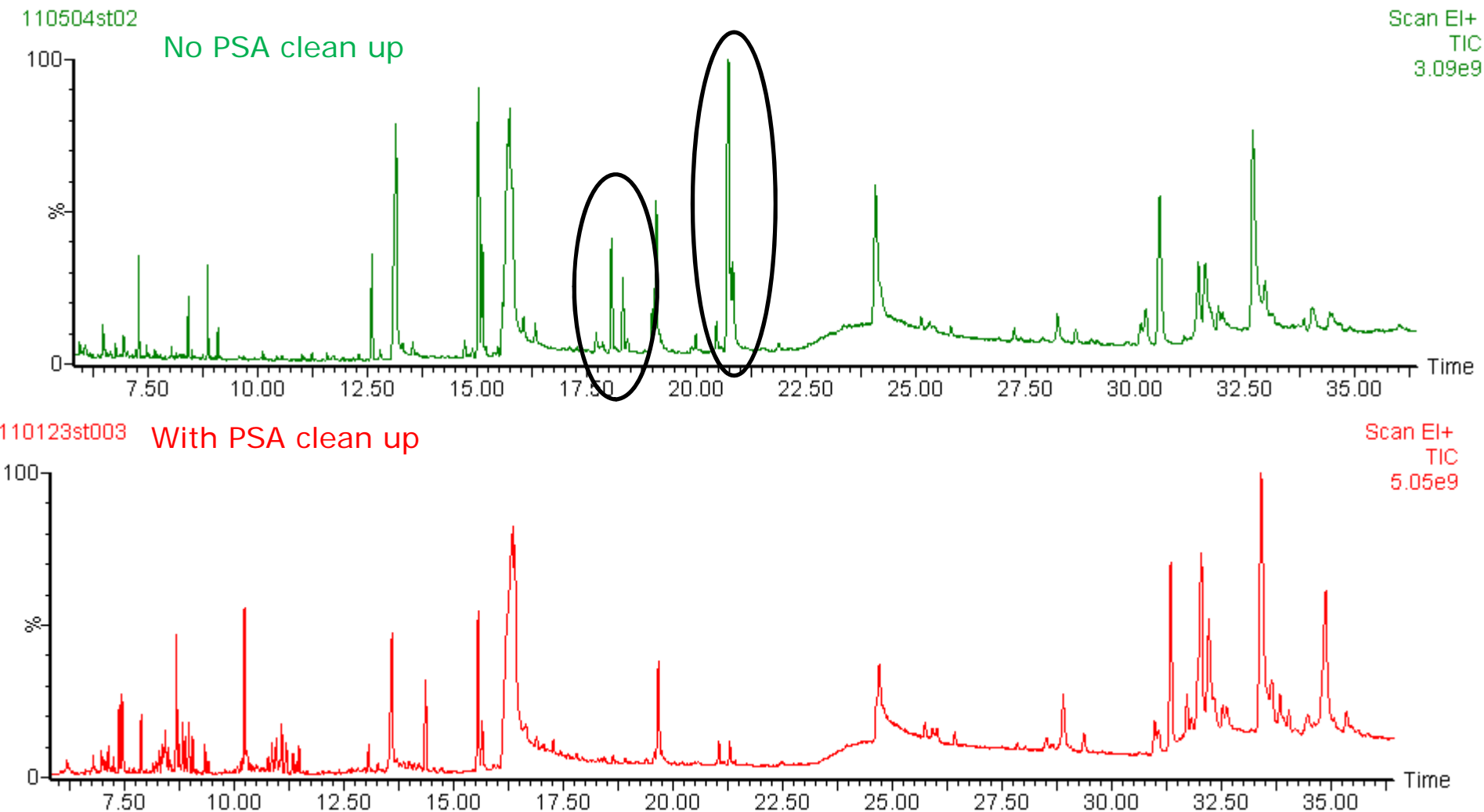
# Freeze out step, -80 °C in one hour



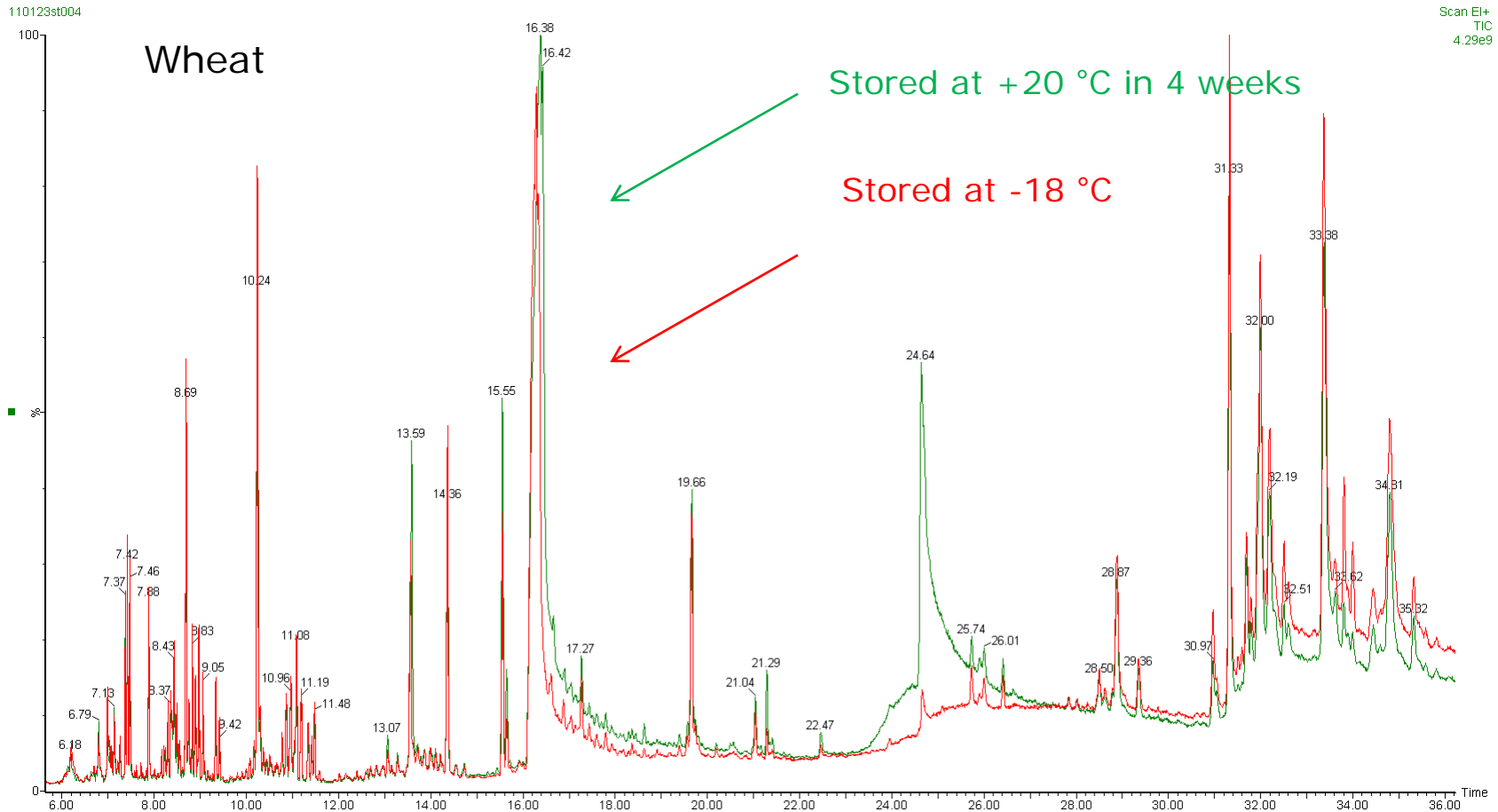
# Freeze out step, -80 °C in one hour



# PSA clean-up - wheat

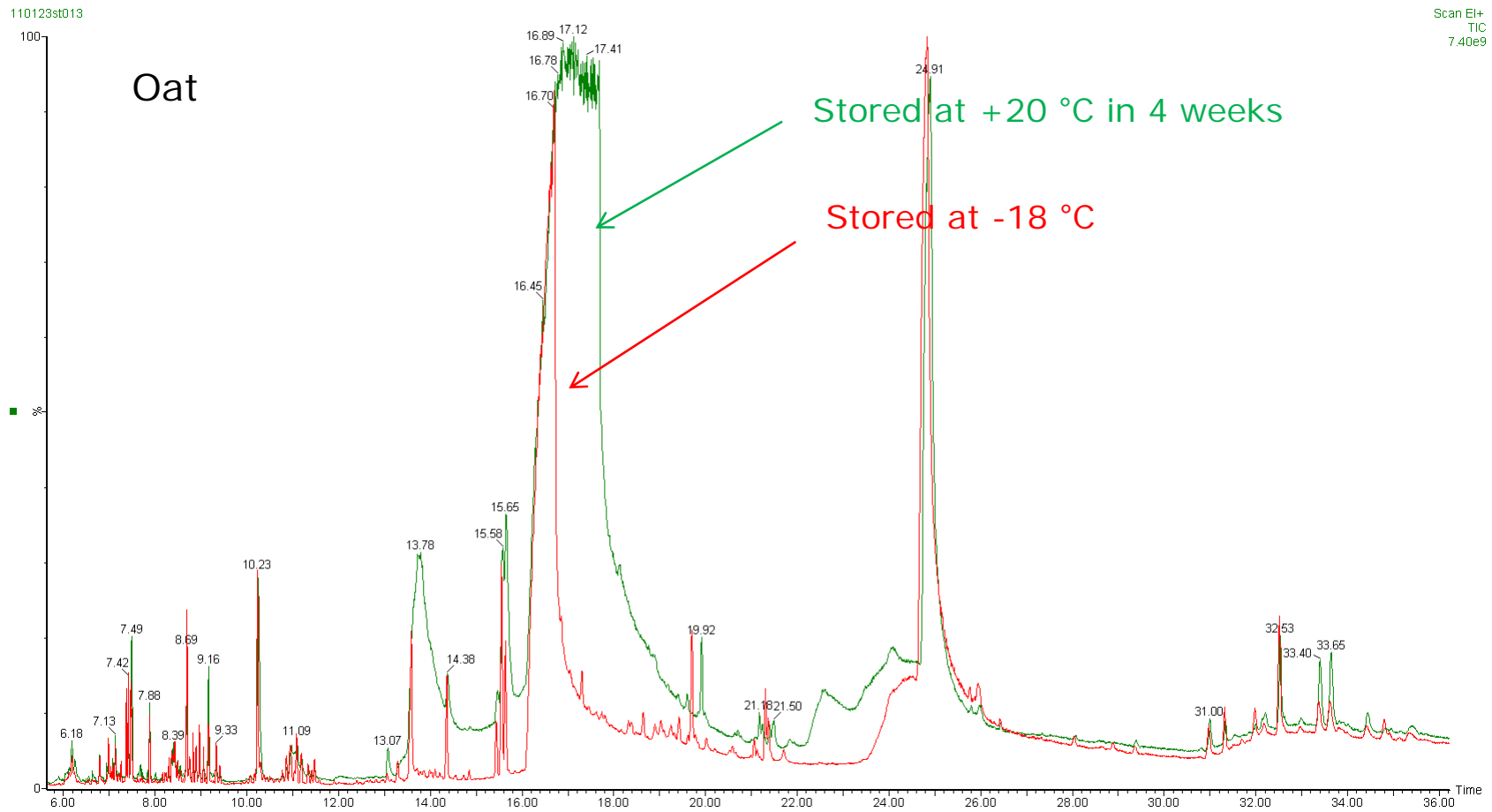


# Storage temperature of samples - Freezer or room temperature

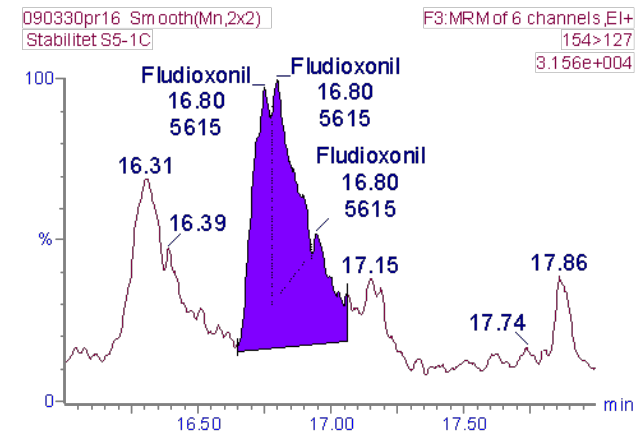
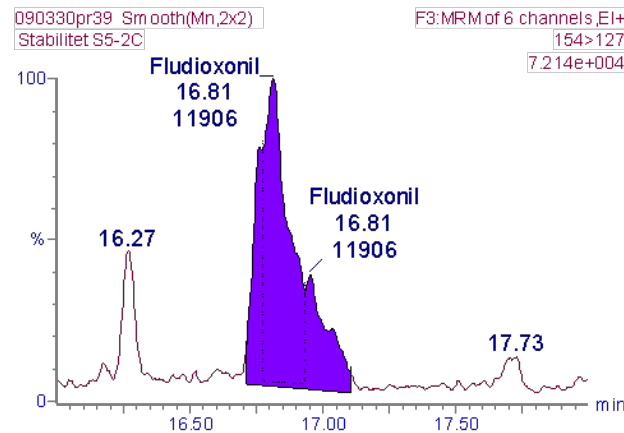
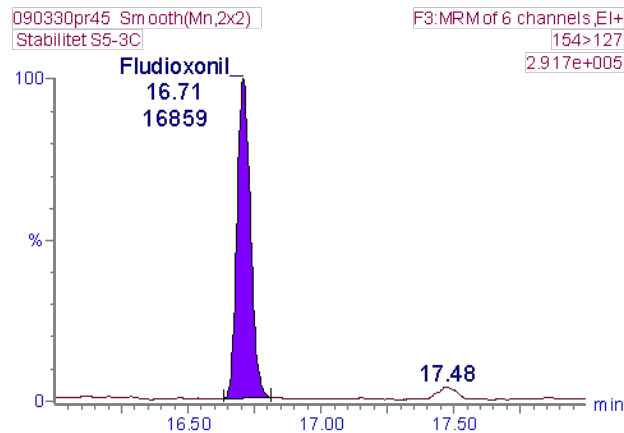
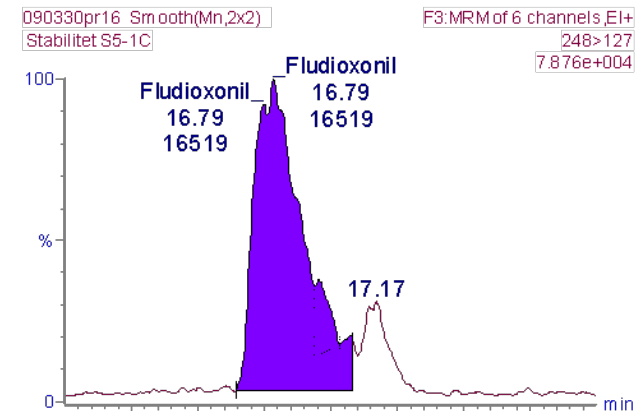
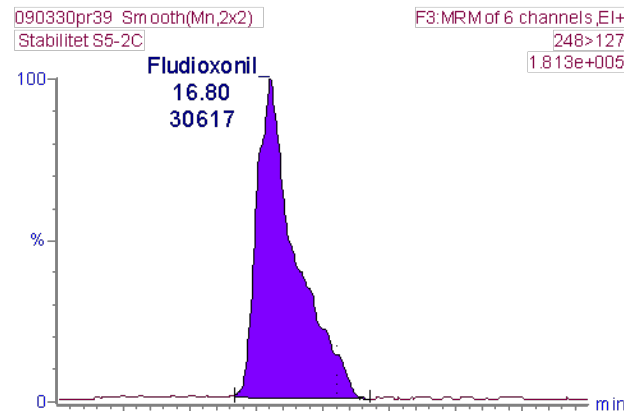
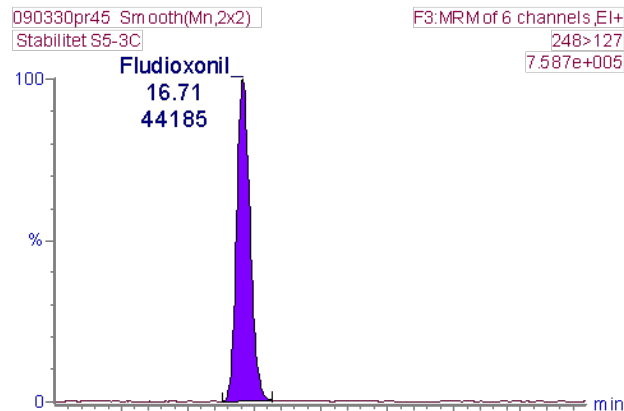


# Storage temperature of sample

## Freezer or roomtemperature



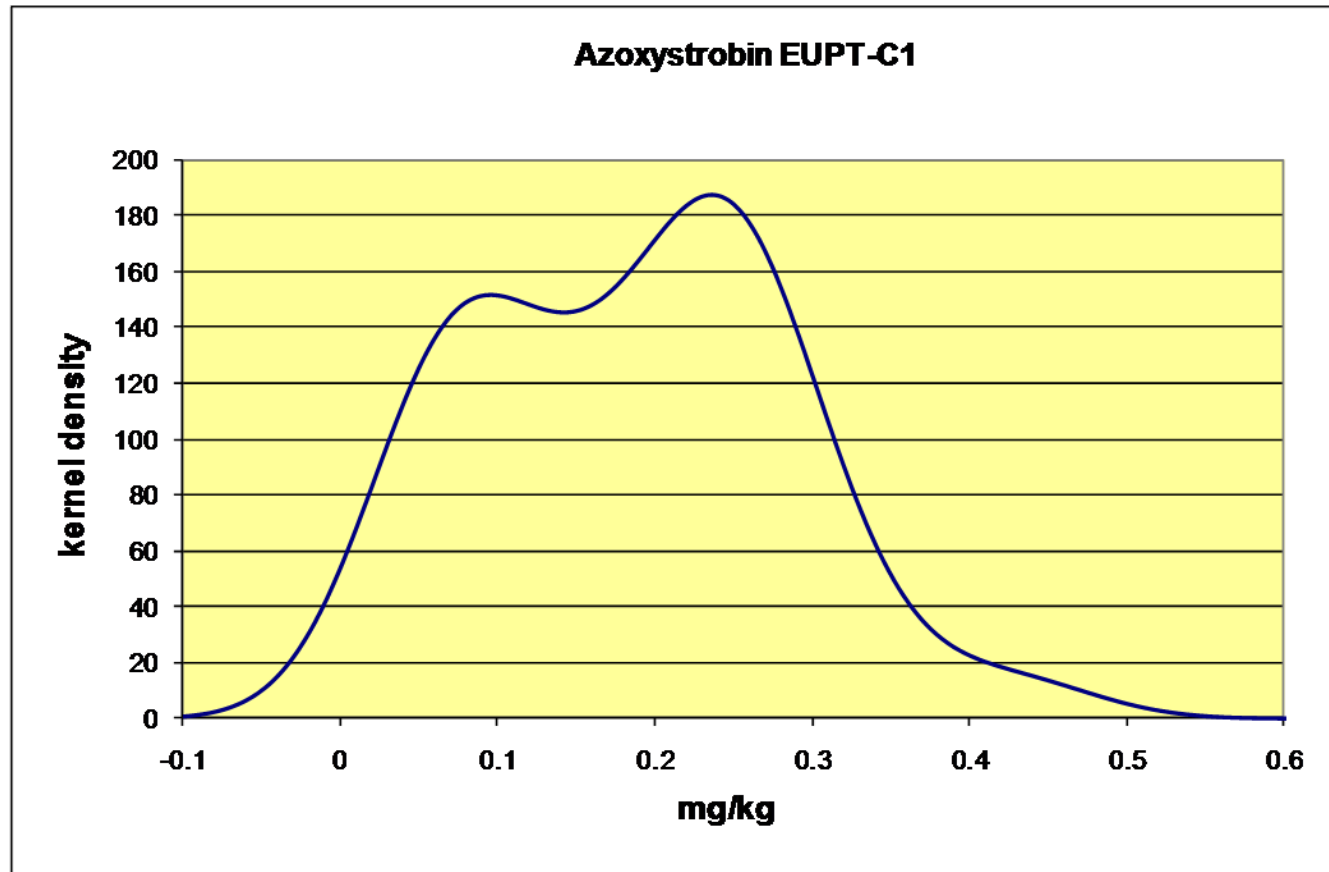
# Stability test – EUPT-C3 (oat, Fludioxonil)



# Recommendation

- 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  $-18^{\circ}\text{C}$

# 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

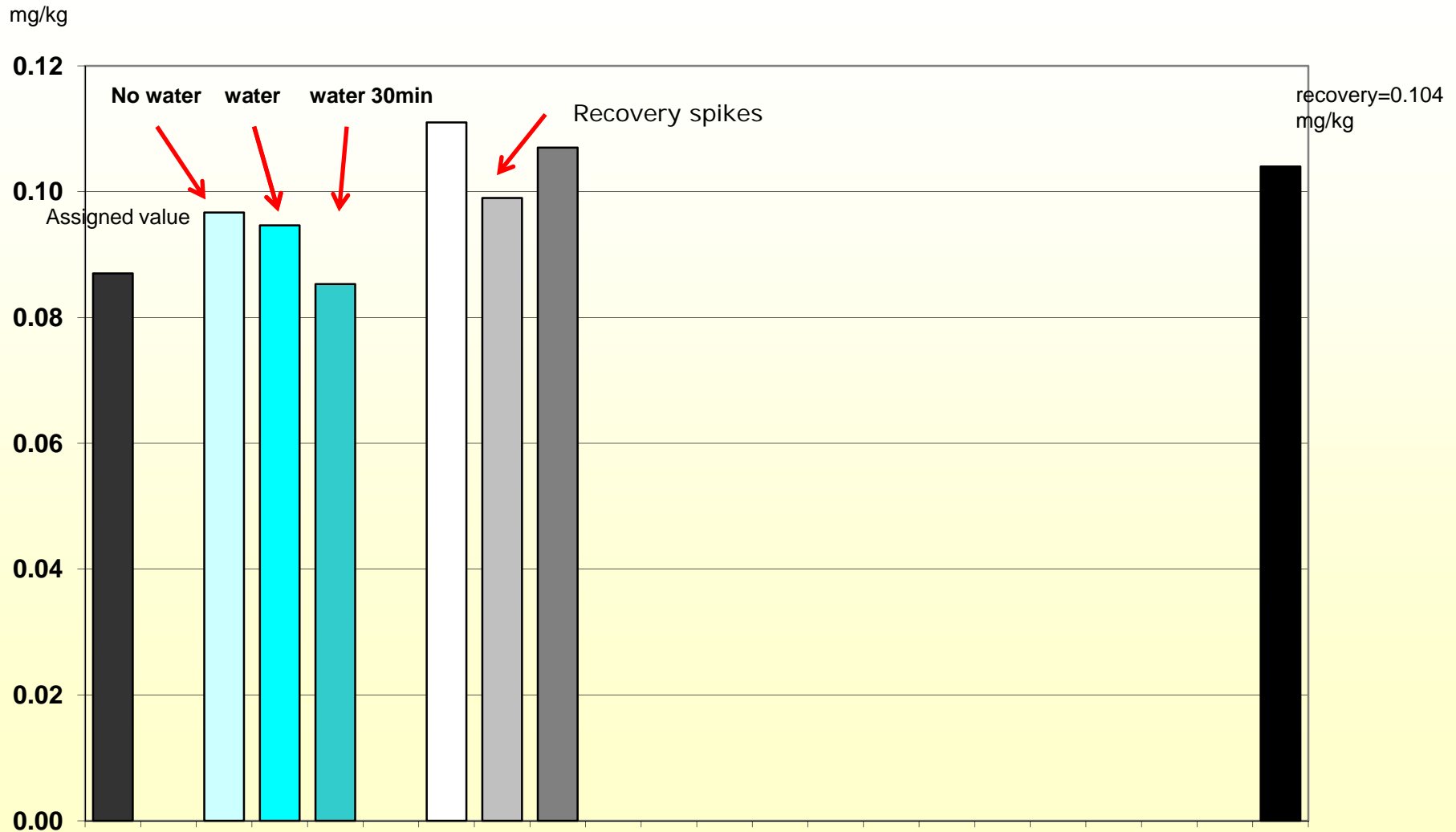
Azoxystrobin PTC1	0.189	0.240	0.074	3.2
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# Experiments on water addition

- 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**

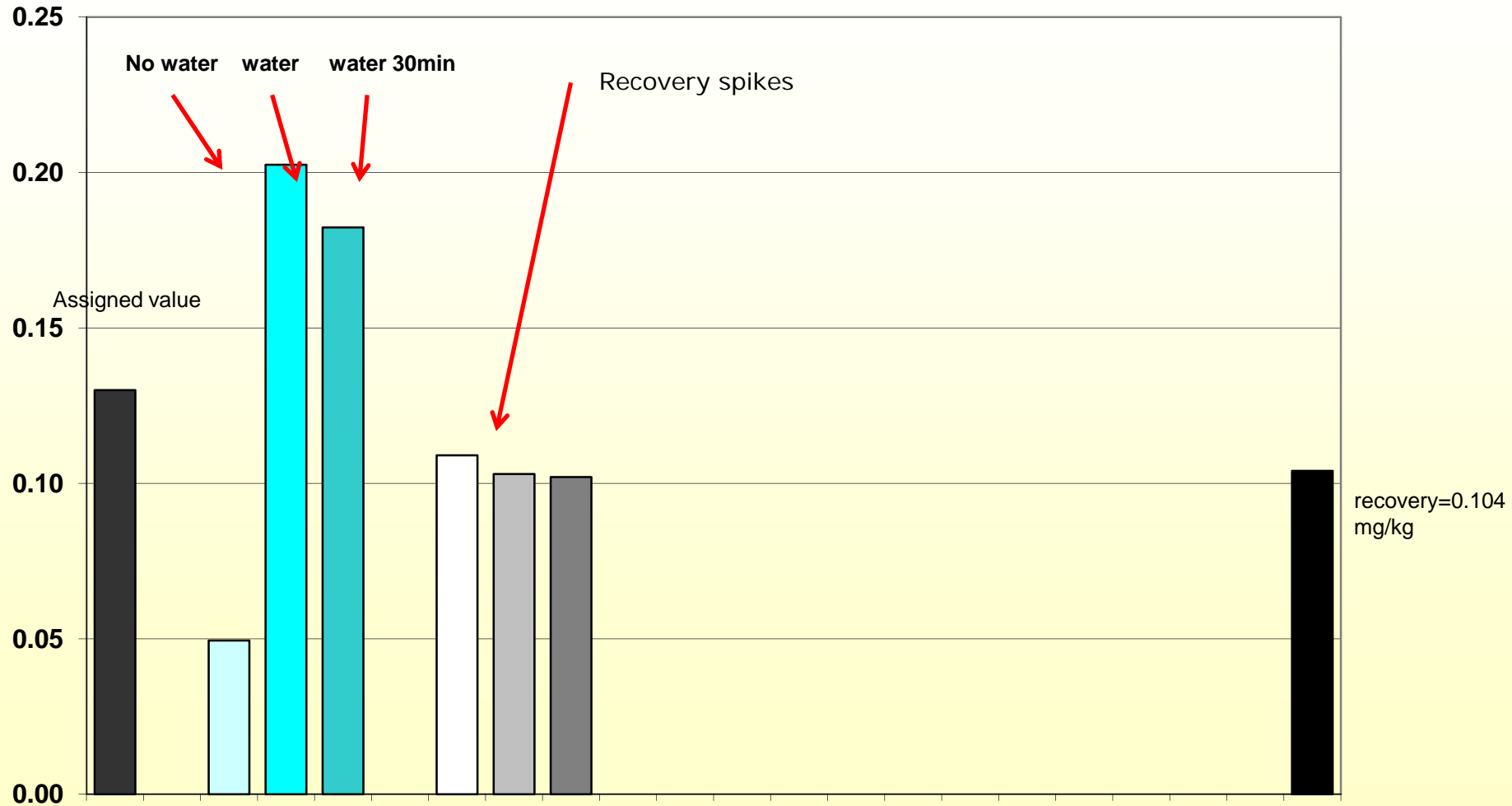
PTC2 - incurred



# Chlorpyrifos-methyl

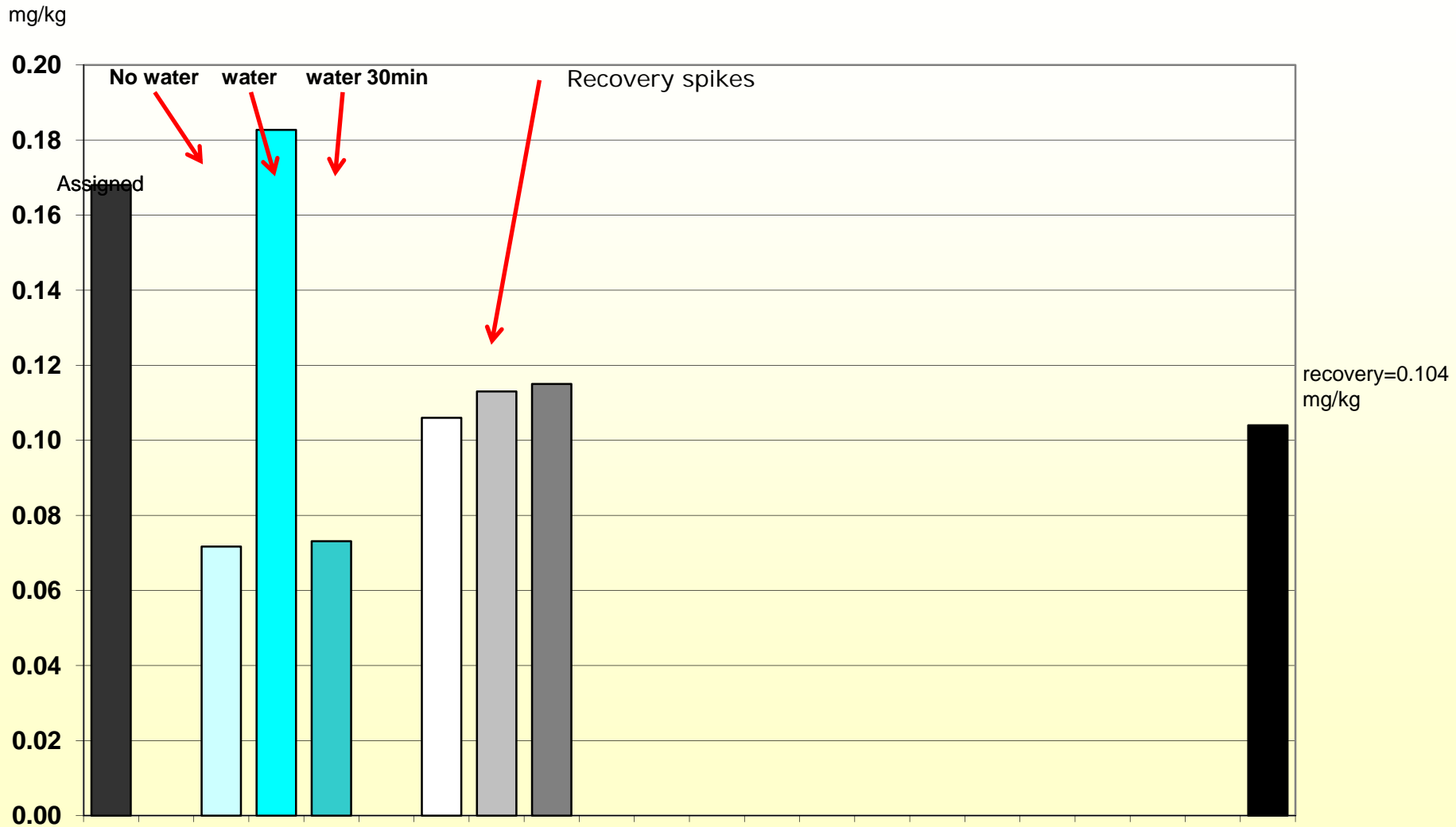
PTC2 - incurred

mg/kg



# Malathion

PTC2 - spiked



# Malation

- Yoshii et al, J. Agric. Food Chem. (2000): *Degradation of malathion and phenthoate by glutathione reductase in wheat germ*
- Yoshii et al, J. Health Science (2006): *Malathion residue in wheat kernels is degraded by thion OPP-specific carboxylesterase*
- Yoshii et al, 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*

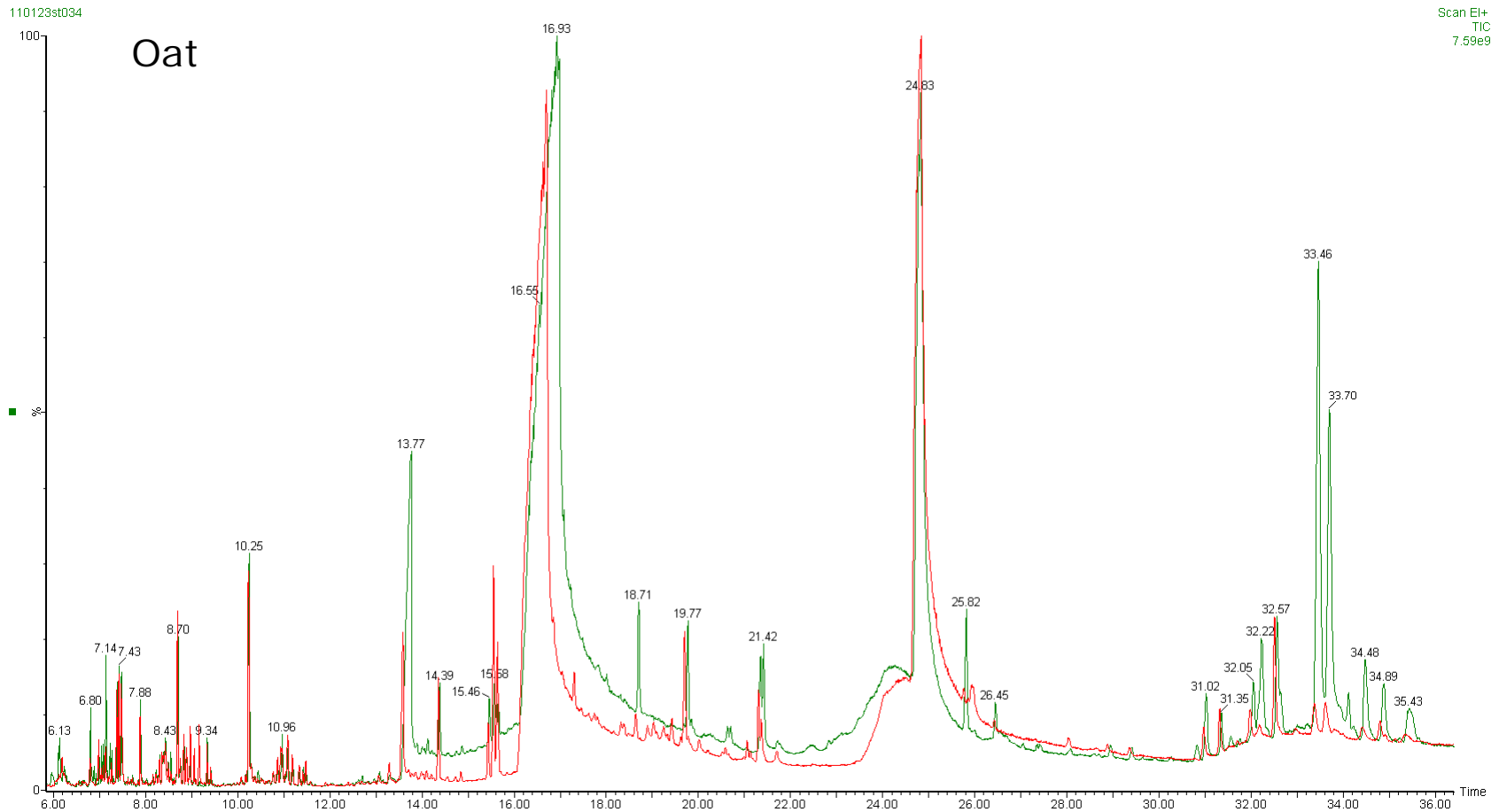
# Recommendations in Document No. SANCO/10684/2009

- 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.

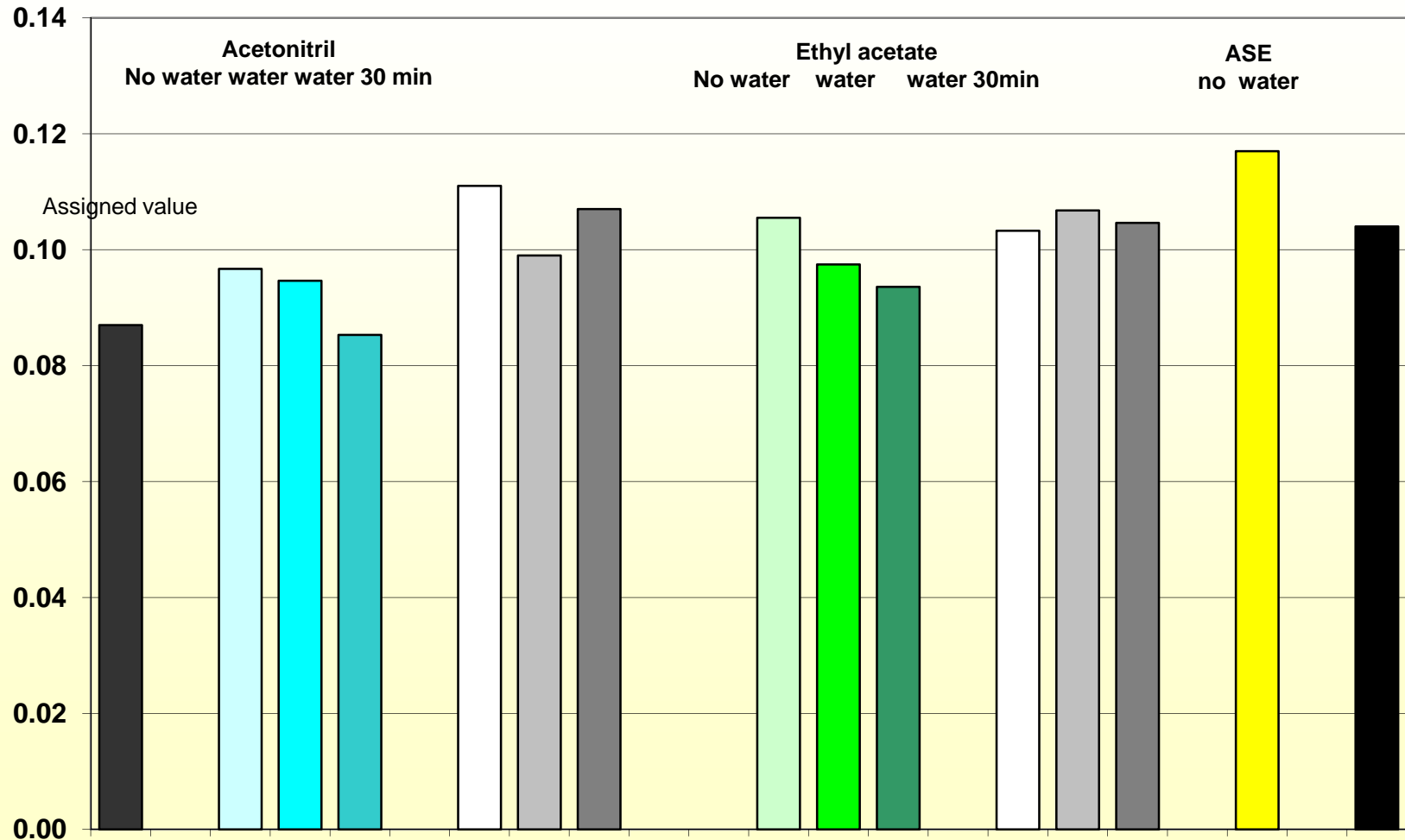
# Acetonitril and ethyl acetate



# Bifenthrin

PTC2 - incurred

mg/kg

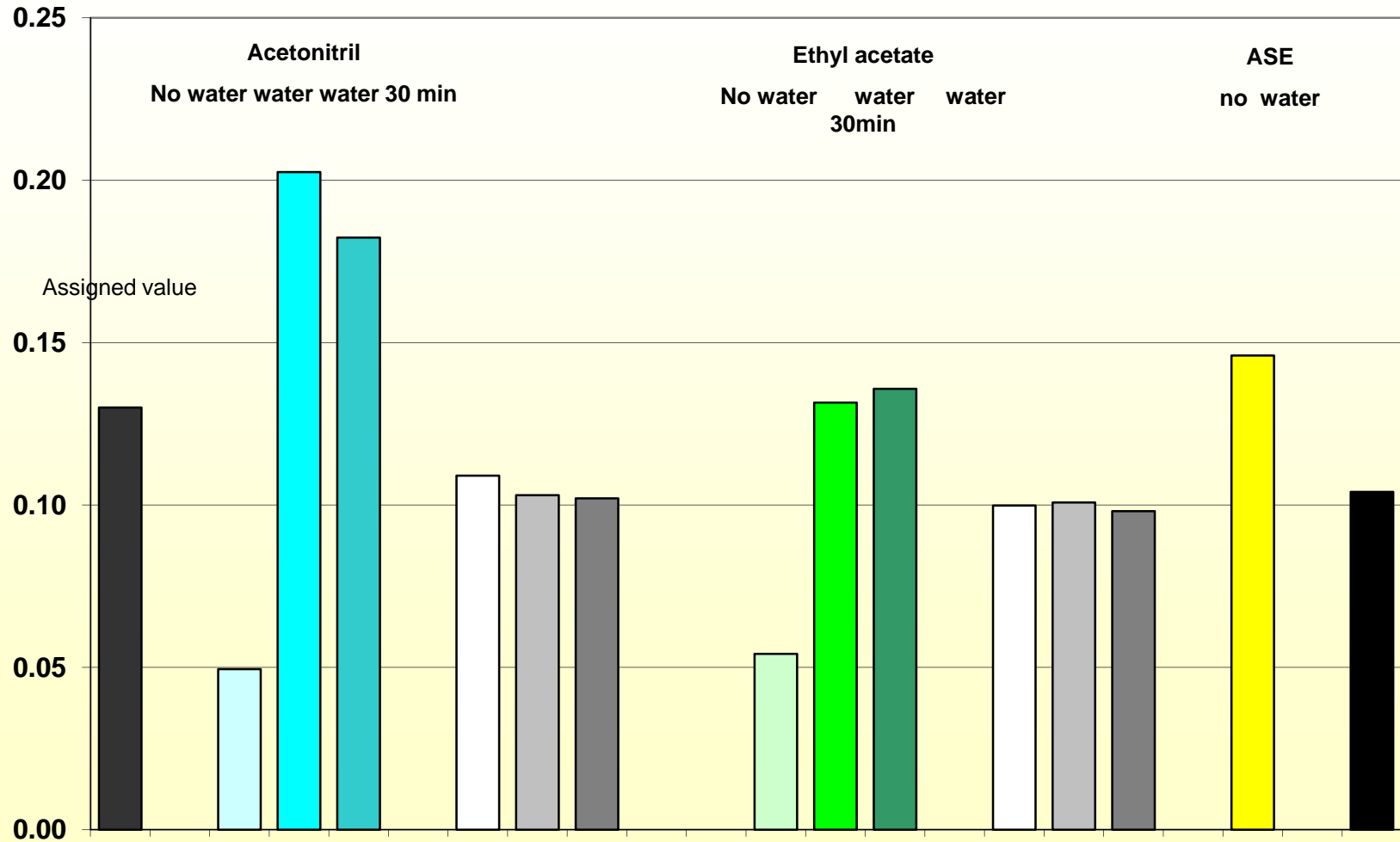


recovery=0.104  
mg/kg

# Chlorpyrifos-methyl

PTC2 - incurred

mg/kg

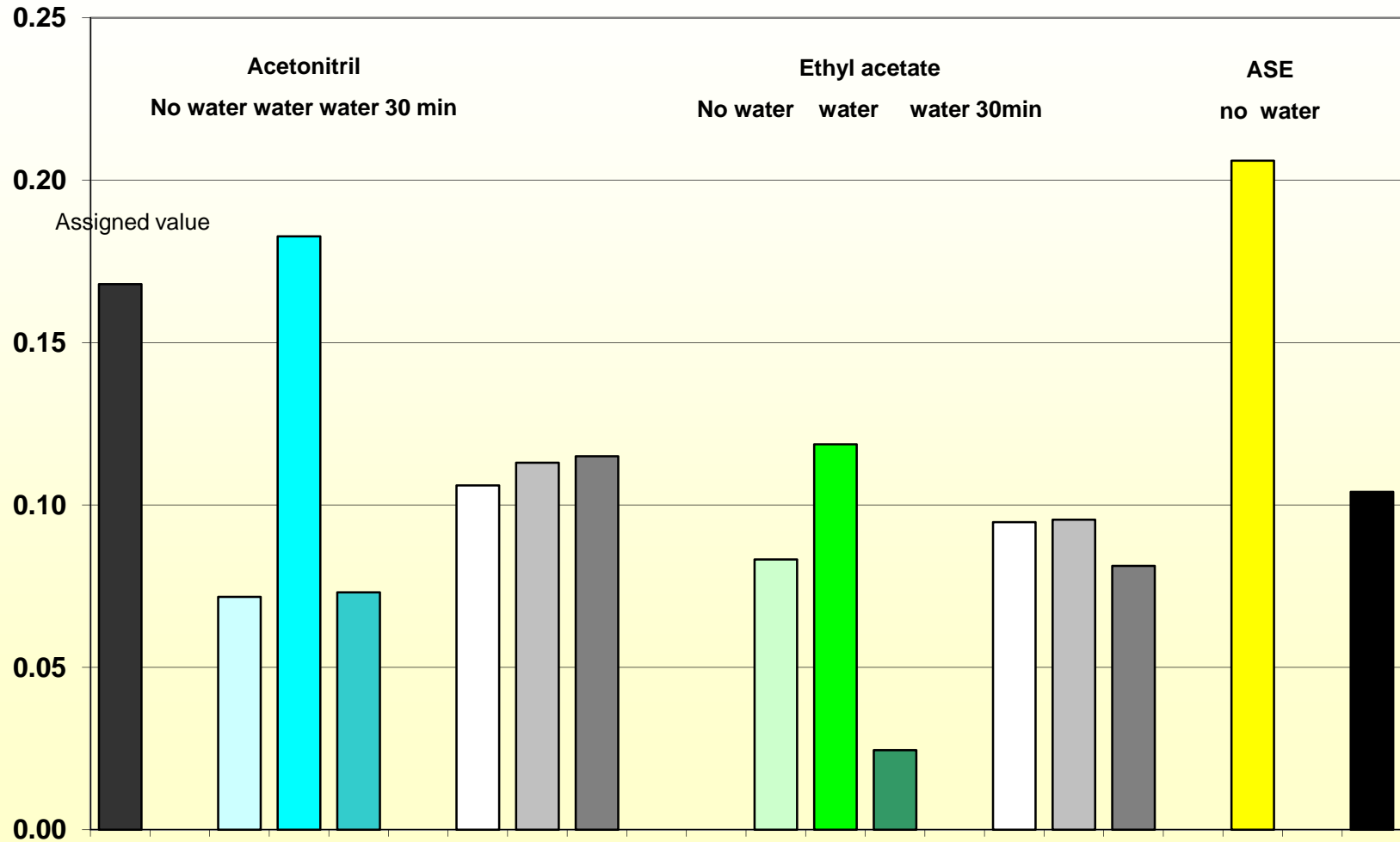


recovery=0.104  
mg/kg

# Malathion

PTC2 - incurred

mg/kg



recovery=0.104  
mg/kg

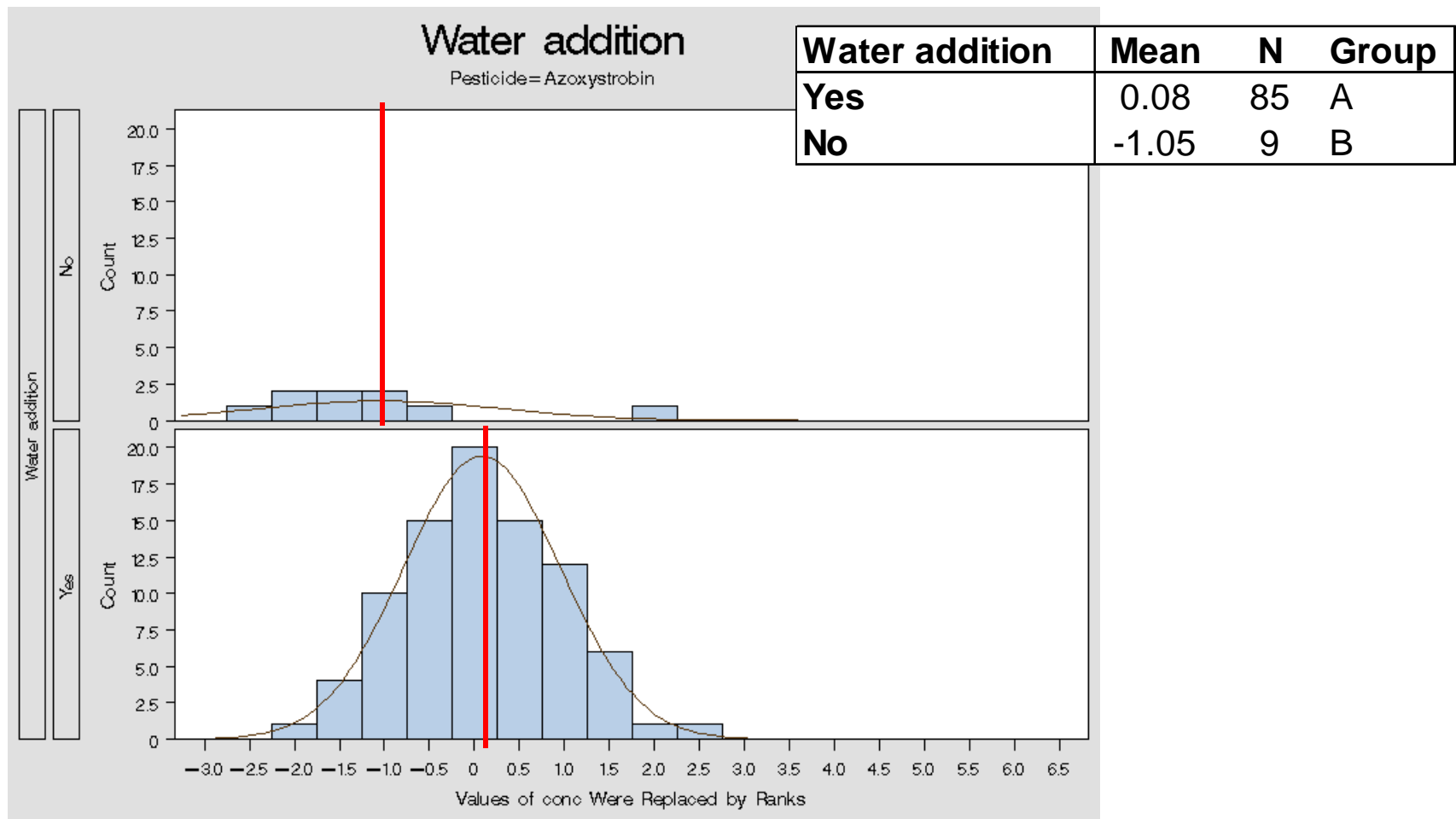
# Results from Proficiency test EUPT-C4

- 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

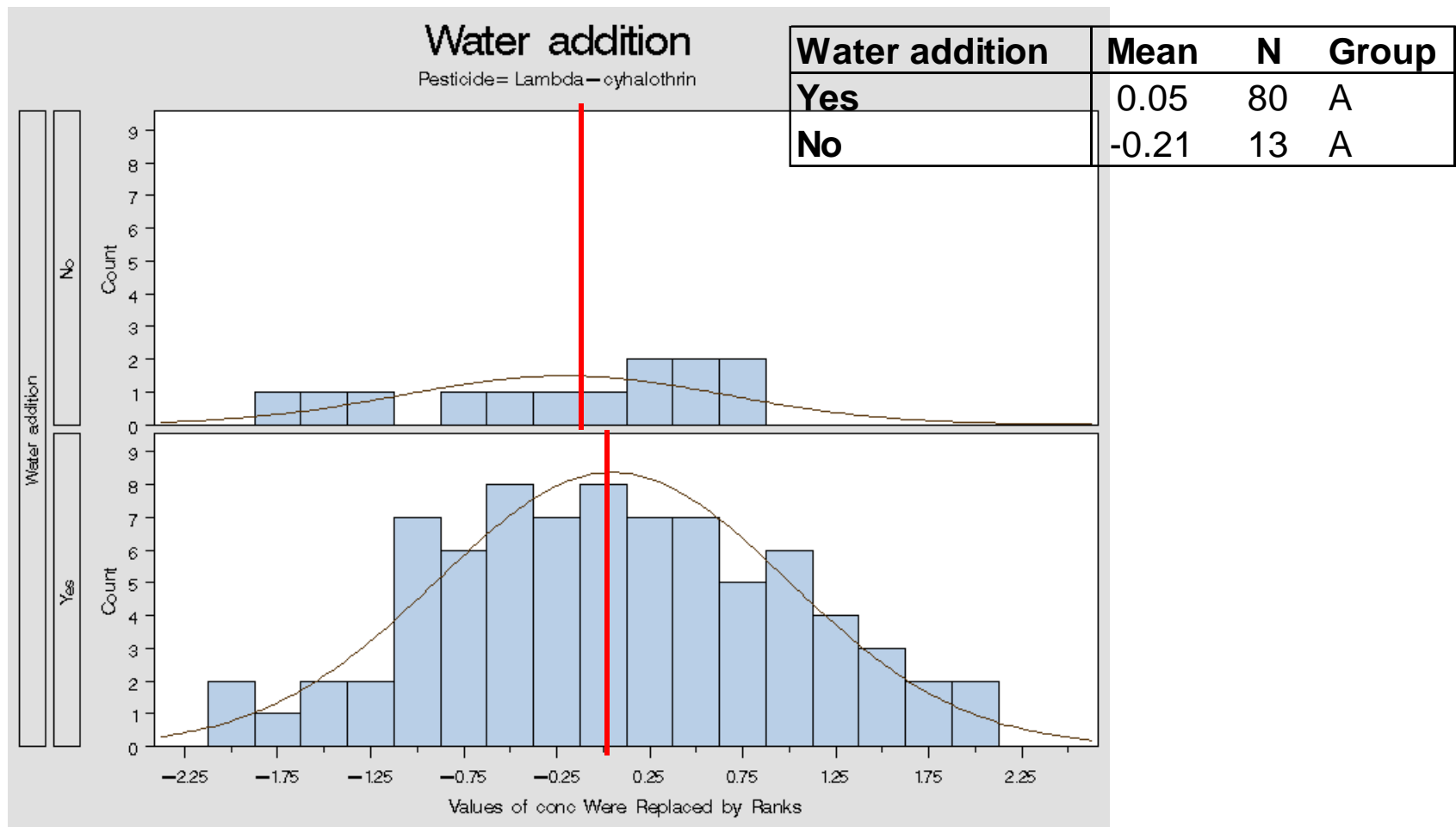
# Pesticides - concentration – by Water Addition

- Differences between water added yes/no

# Azoxystrobin



# Lambda-cyhalothrin

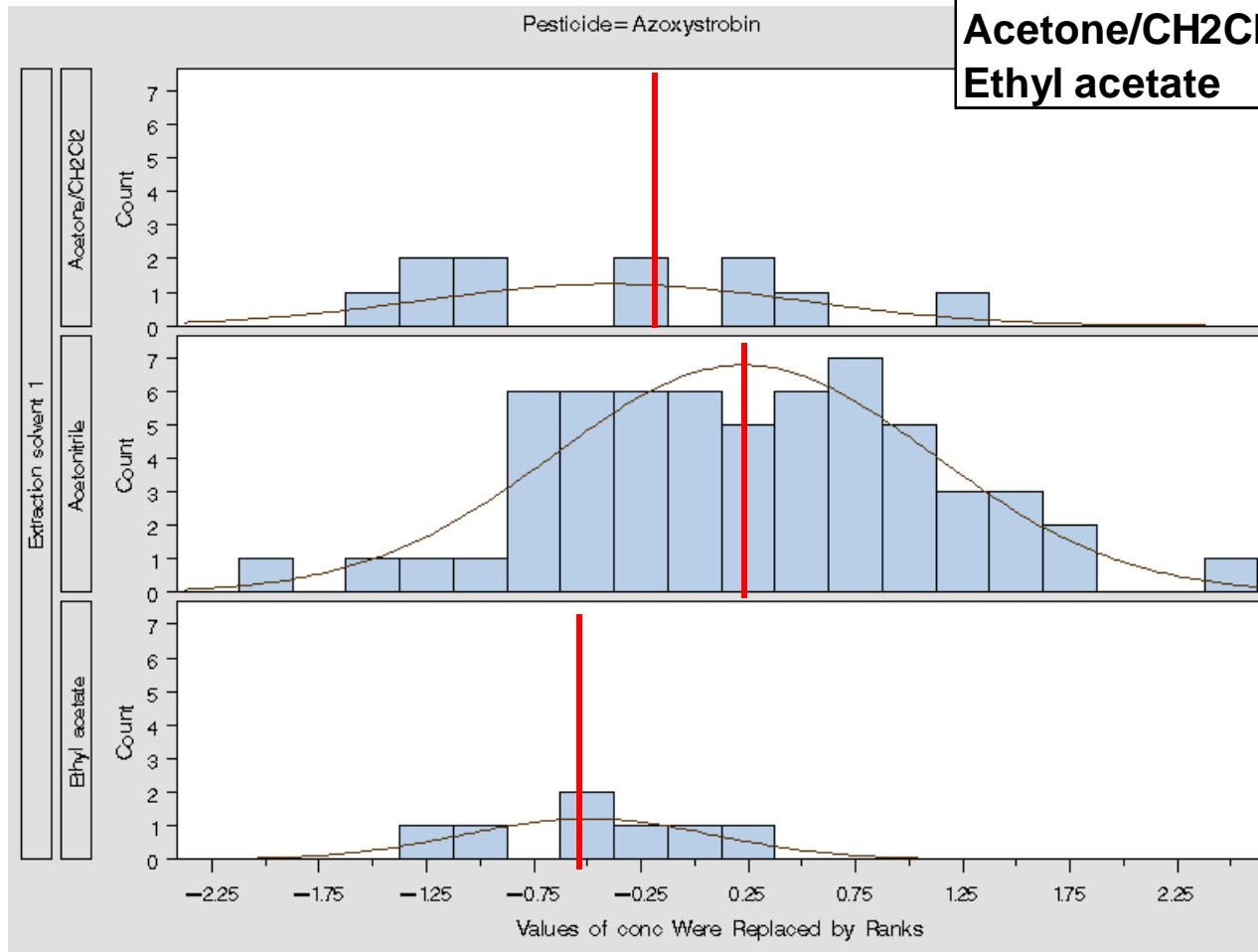


# Pesticides - concentration – by Extraction Solvent 1

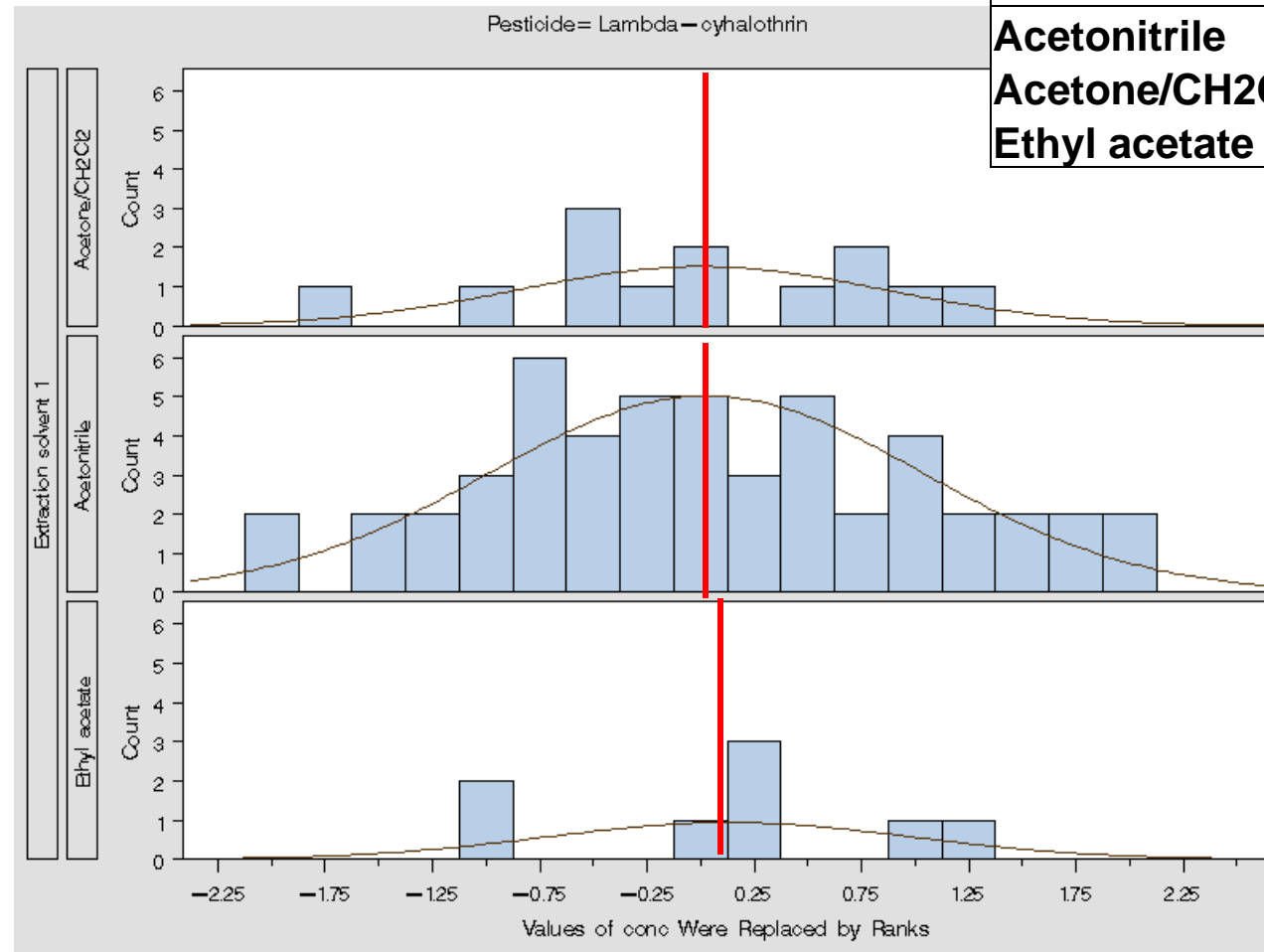
- Differences between extraction solvents
  - Acetonitril
  - Acetone/ $\text{CH}_2\text{Cl}_2$
  - Ethyl acetate

# Azoxystrobin

Solvent	Mean	N	Group
Acetonitrile	0.23	60	A
Acetone/CH <sub>2</sub> Cl <sub>2</sub>	-0.39	11	AB
Ethyl acetate	-0.50	7	B



# Lambda-cyhalothrin



Solvent	Mean	N	Group
Acetonitrile	0.03	60	A
Acetone/CH <sub>2</sub> Cl <sub>2</sub>	0.01	11	A
Ethyl acetate	0.13	7	A

# Two sided analysis of variance

- Factor 1: solvent

Acetone	7
Acetone/CH <sub>2</sub> Cl <sub>2</sub>	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 dropped, due to degradation
- Statistical 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