Automatic sample preparation

Ederina Ninga

European Reference Laboratory for Cereal and Feed
Research Group of Analytical Food Chemistry, DTU-Food, Denmark
edni@food.dtu.dk

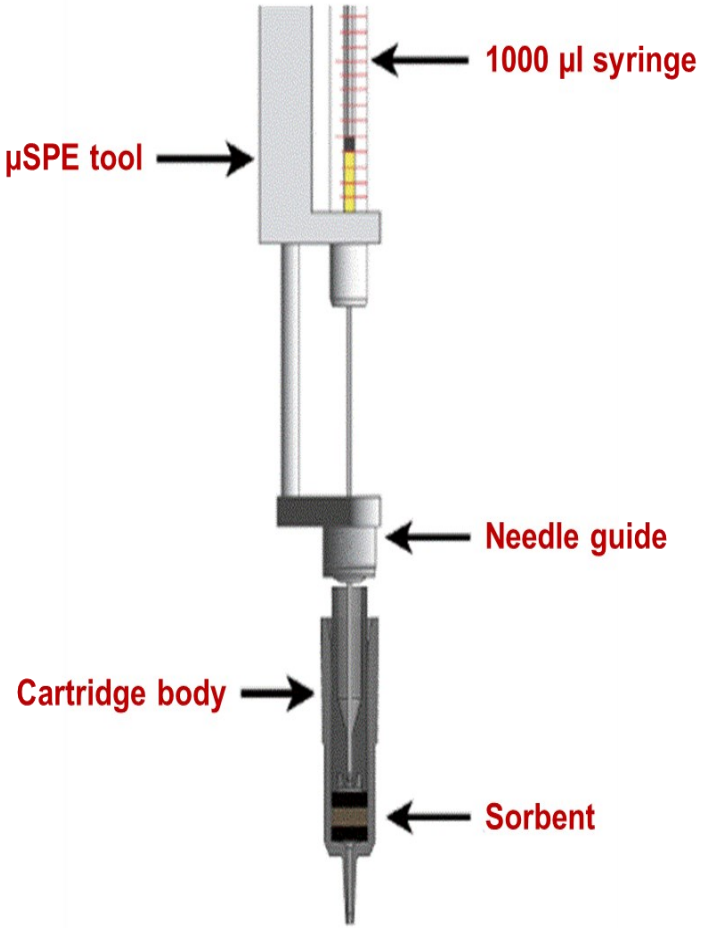
2021 – Future work

- Extending our validation scope in more difficult matrices using the µSPE clean-up.
- Transferring the knowledge.
- Finalizing the automatic calibration curve.
- ……

2021 – Future work

- **Extending our validation scope in more difficult matrices using the µSPE clean-up.**
- **Transferring the knowledge.**
- **Finalizing the automatic calibration curve.**

Automated µSPE clean-up



ITSP cartridge

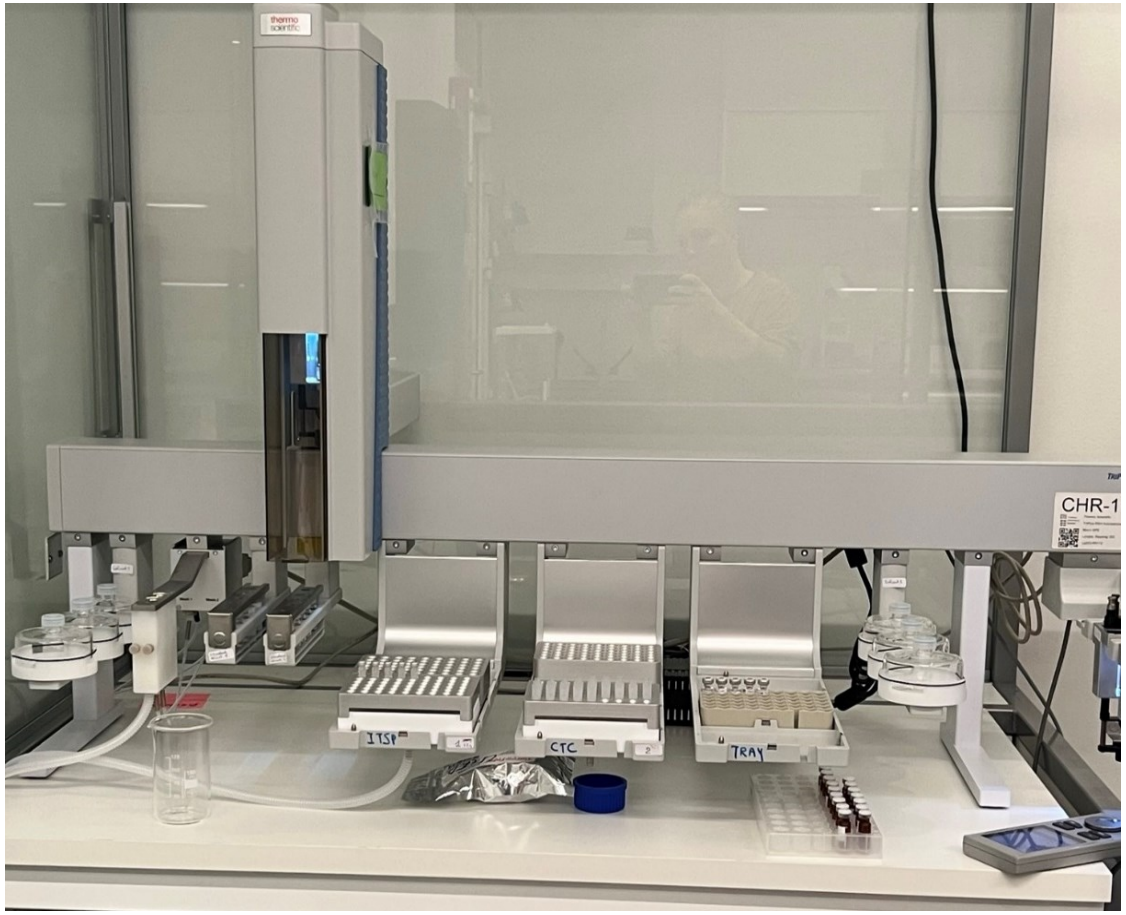
PAL System µSPE cartridges

Automated µSPE clean-up

Instrument Top Sample Preparation

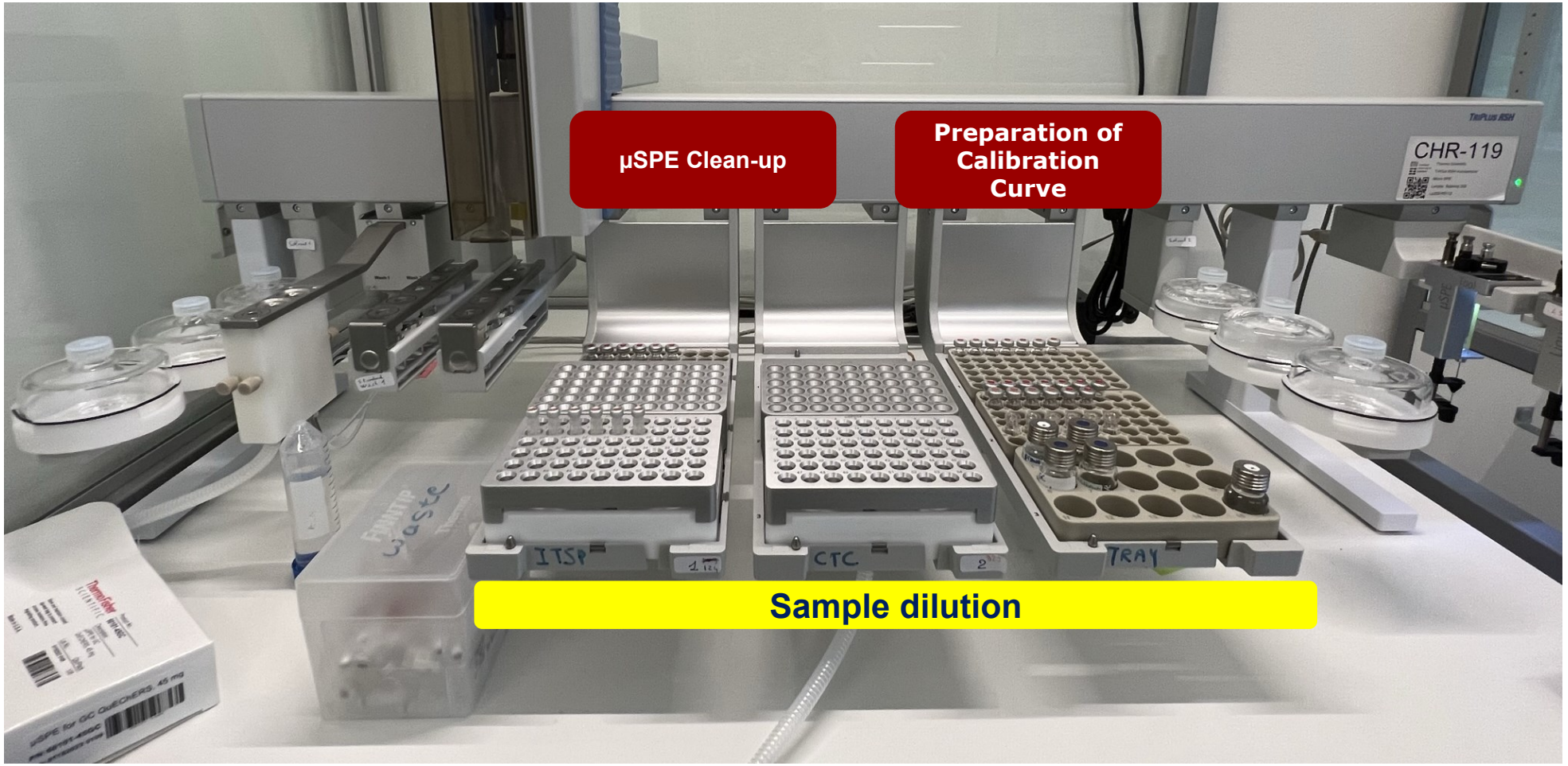
Source: Lehotay, S. J.
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center; 600 East Mermaid Lane; Wyndmoor, PA 19038; USA

Stand Alone Multi-purpose Autosampler



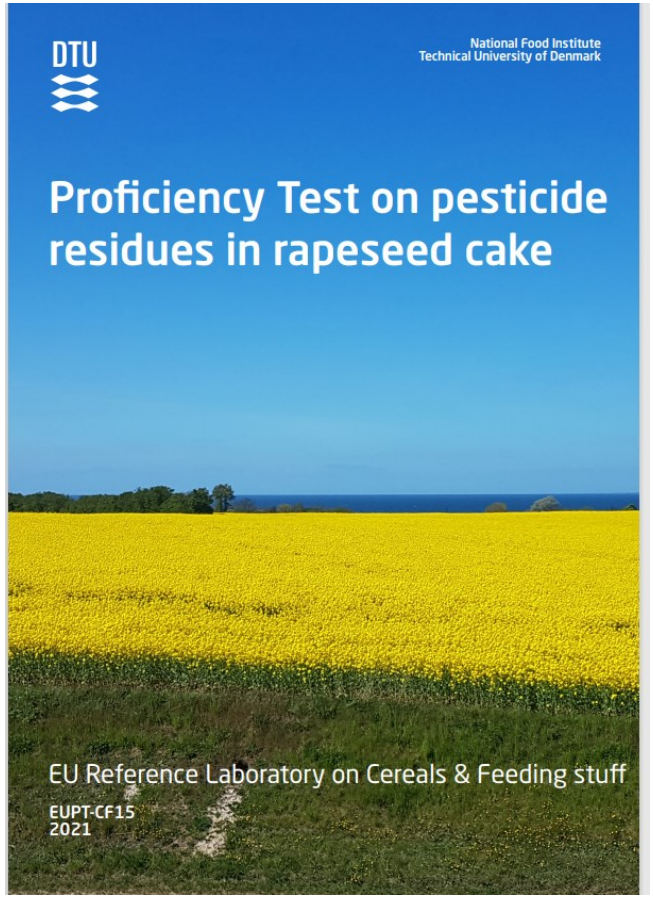
National Food Institute, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark

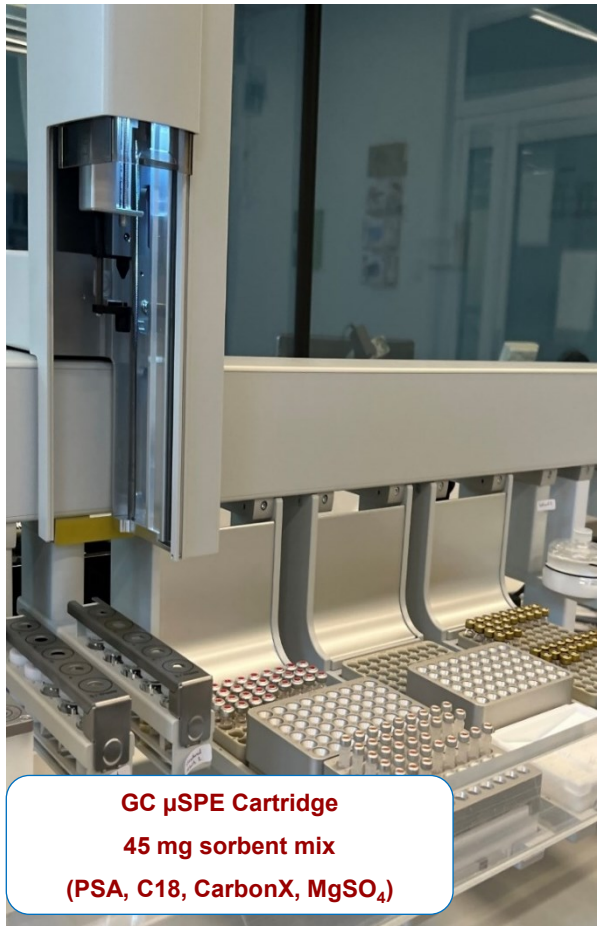
Stand Alone Multi-purpose Autosampler



Extending our validation scope in more difficult matrices using the µSPE clean-up

Rapeseed cake





GC µSPE Cartridge

45 mg sorbent mix

(PSA, C18, CarbonX, $MgSO_4$)

Automated µSPE clean-up Rapeseed cake

Initial Manual Step

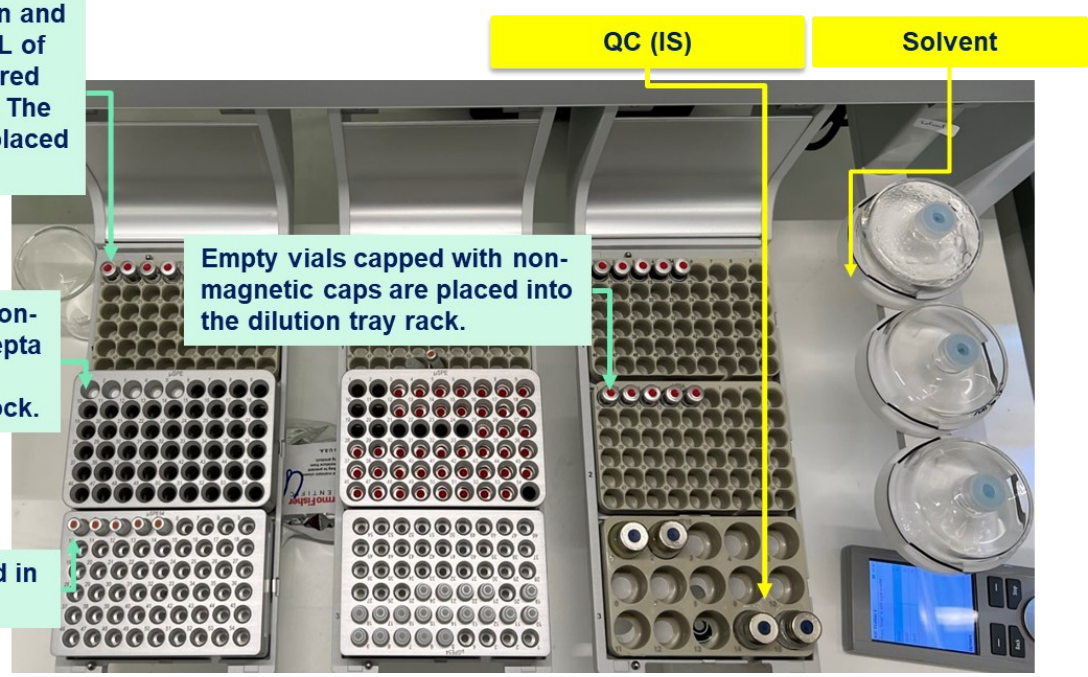
Citrate Buffer QuEChERS (EN 15662)

- 2 g sample
- 10 ml water/ 10 ml acetonitrile extraction
- Salting out/shake/centrifugation
- Transfer 8 ml and place them in -80 / centrifugation

After QuEChERS extraction and centrifugation approx. 1 mL of the supernatant is transferred to 2 mL autosampler vials. The vials with raw extract are placed into the sample rack.

Empty vials capped with non-magnetic caps and split septa are placed into the eluate rack, covered by the vial lock.

µSPE cartridges are placed in the cartridge rack.



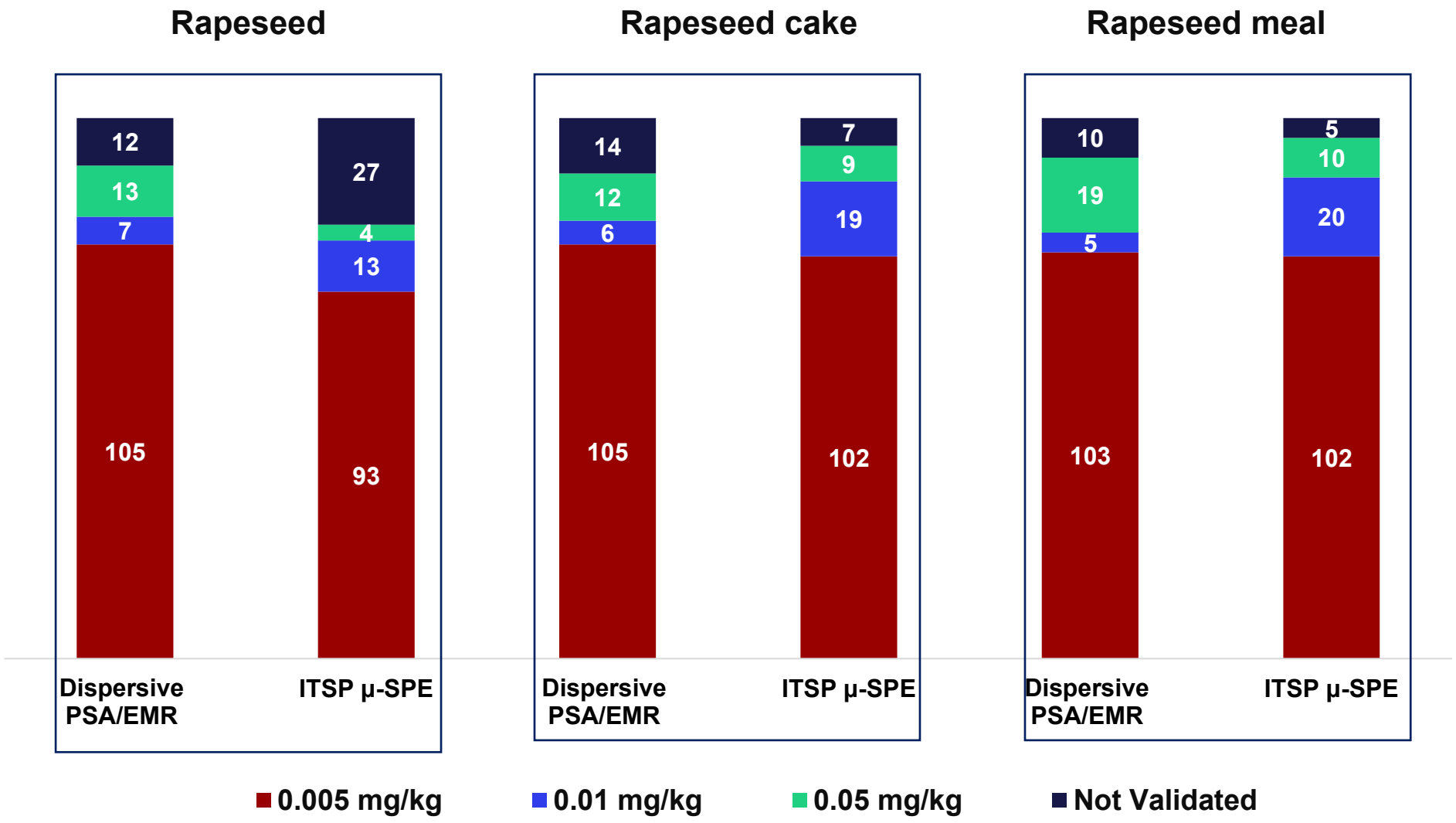
µSPE clean-up

Select the µSPE tool with 1000 µL syringe. → Pull up 300 µL raw extract from the Sample Rack into the 1000 µL syringe. → Transfer the µSPE cartridge by syringe needle to the Elution Rack. → Elute extract through the µSPE cartridge at 2 µL/s. → Discard cartridge into waste receptacle.

Dilution

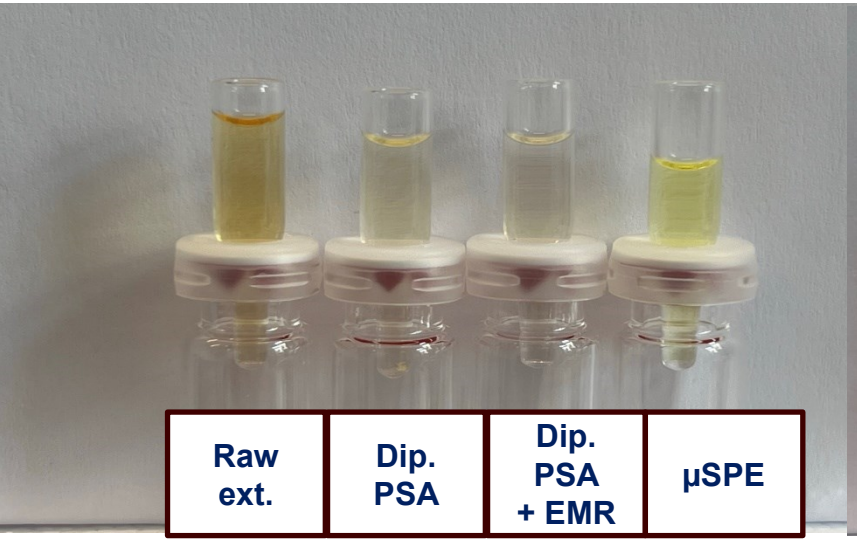
Transfer 100 µL of clean eluate on final vial. → Change the syringe into the 100 µL syringe. → Transfer in the final vial 20 µl of IS (QC) on the final vial. → Change the syringe into the 1000 µL syringe. → Transfer acetonitrile 100 µL (for sample preparation). → Mixing.

Validation of Automated µSPE Extract Clean-up for pesticides in Rapeseeds by GC-MS/MS

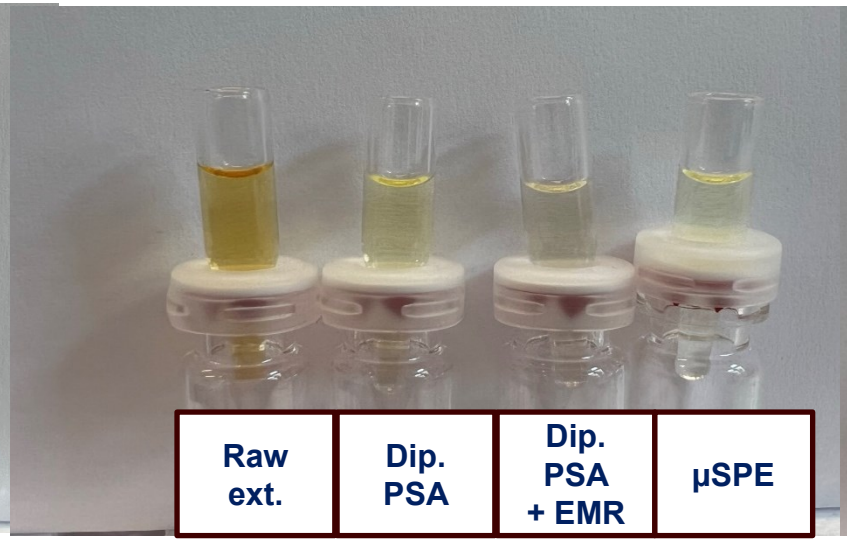


Validation of Automated µSPE Extract Clean-up for pesticides in Rapeseeds by GC-MS/MS

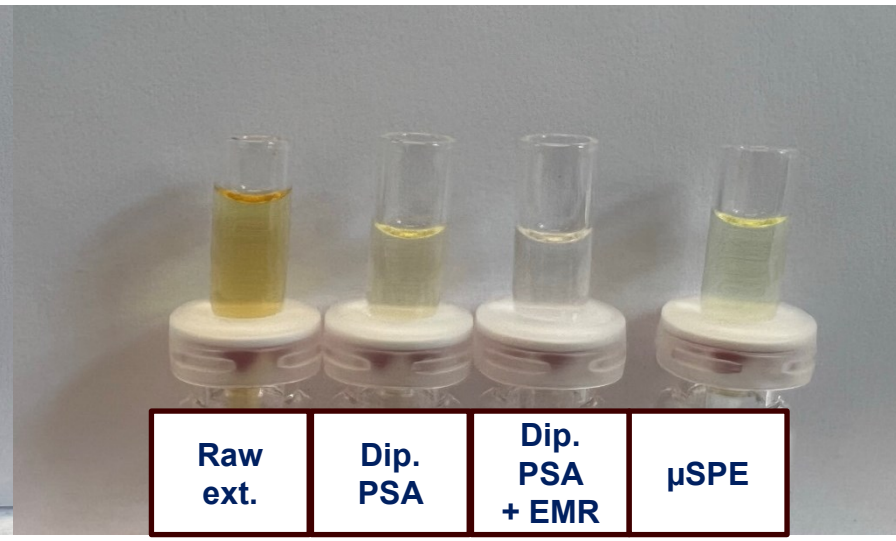
Rapeseed



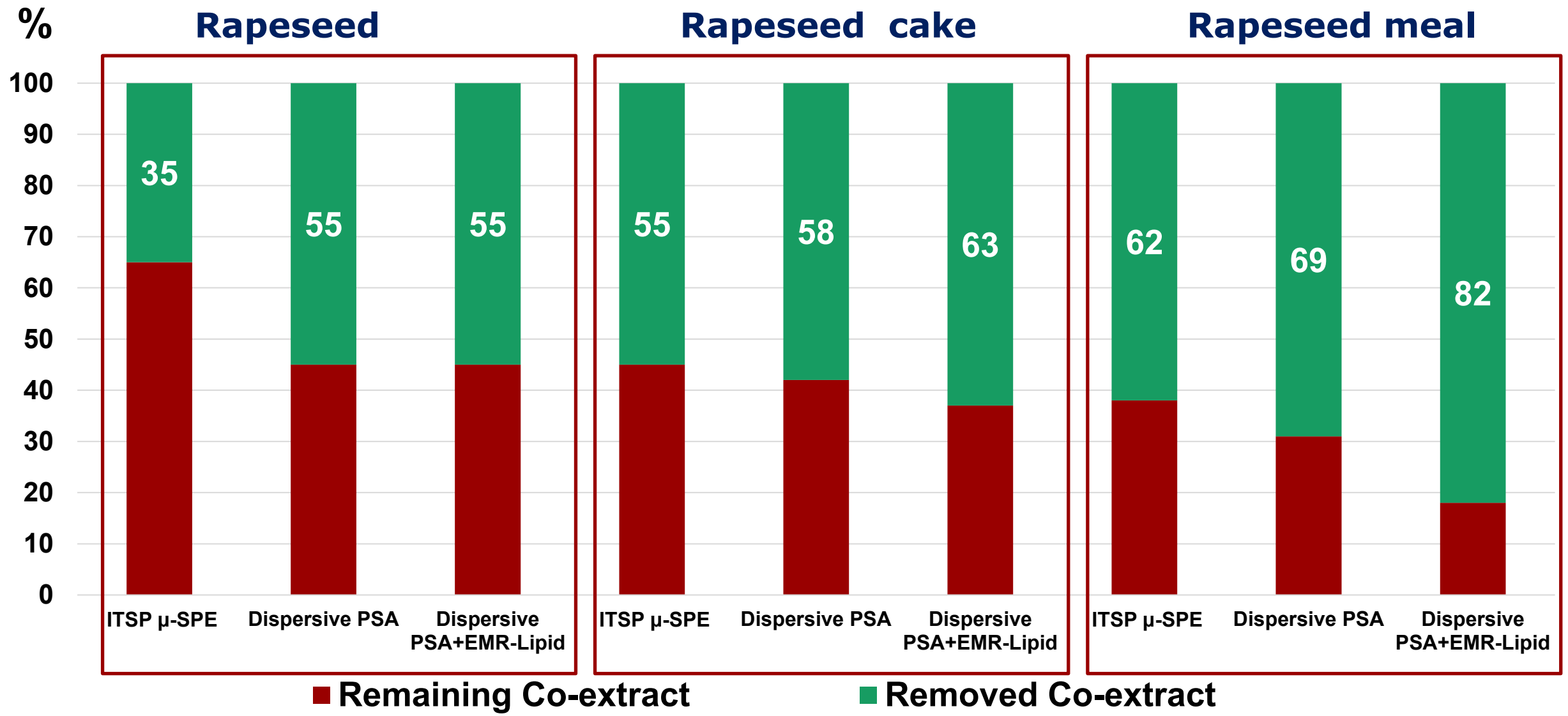
Rapeseed cake



Rapeseed meal



Validation of Automated µSPE Extract Clean-up for pesticides in Rapeseeds by GC-MS/MS



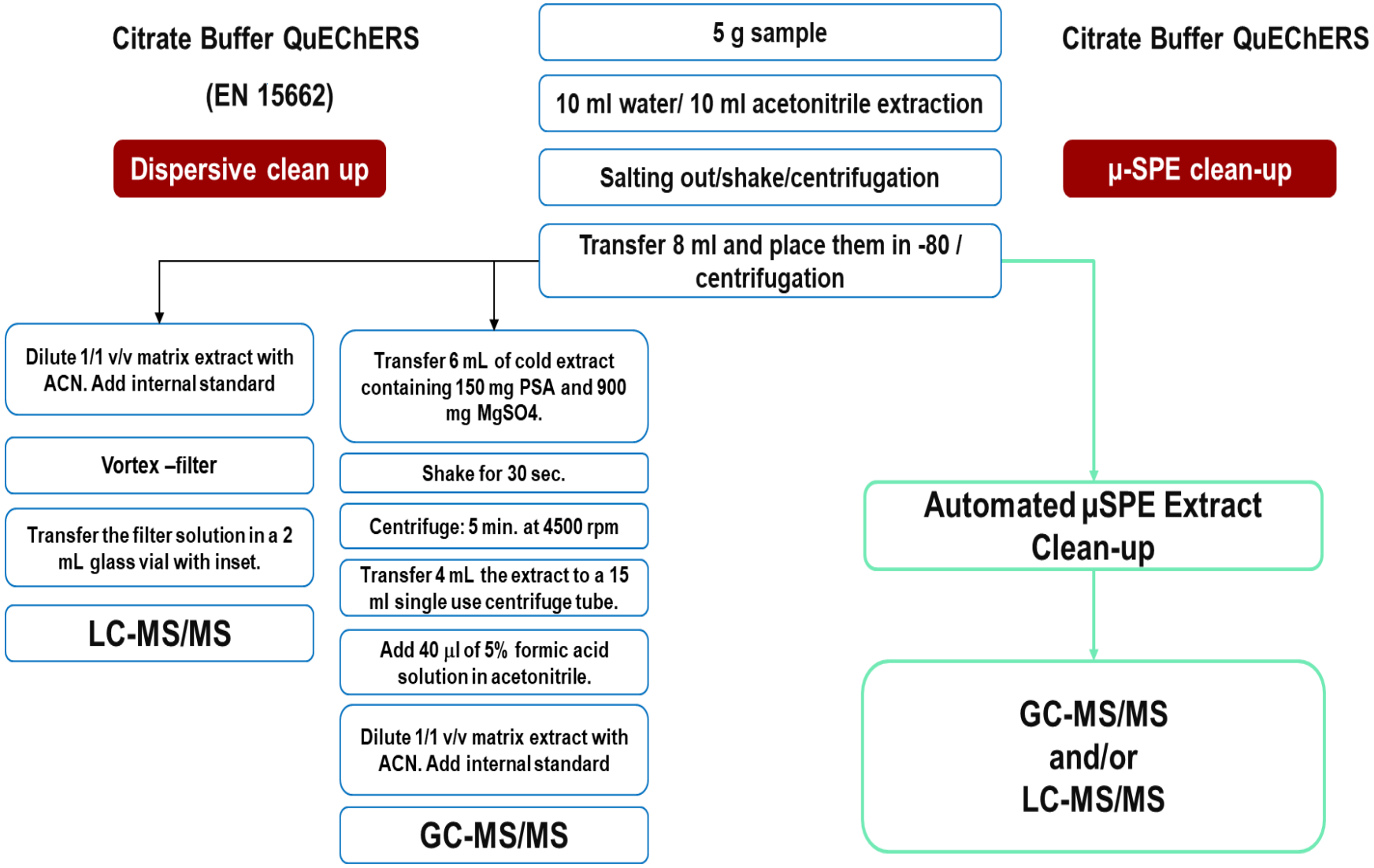
EURL CF-15 Rapeseed cake

Analytes	Assigned value mg/kg	Clean up	CF-15 results mg/kg	Z-score
Azoxystrobin	0.04	PSA/EMR	0.034	-0.80
		µSPE clean up	0.042	-0.01
Boscalid	0.11	PSA/EMR	0.097	-0.32
		µSPE clean up	0.110	0.21
Clomazone	0.08	PSA/EMR	0.074	-0.10
		µSPE clean up	0.070	-0.29
Pendimethalin	0.04	PSA/EMR	0.028	-1.21
		µSPE clean up	0.033	-0.74
Pirimicarb	0.06	PSA/EMR	0.058	-0.04
		µSPE clean up	0.063	0.27
Tebuconazole	0.07	PSA/EMR	0.069	-0.05
		µSPE clean up	0.070	-0.01
Tetraconazole	0.02	PSA/EMR	0.020	0.08
		µSPE clean up	0.023	0.61

2021 – Future work

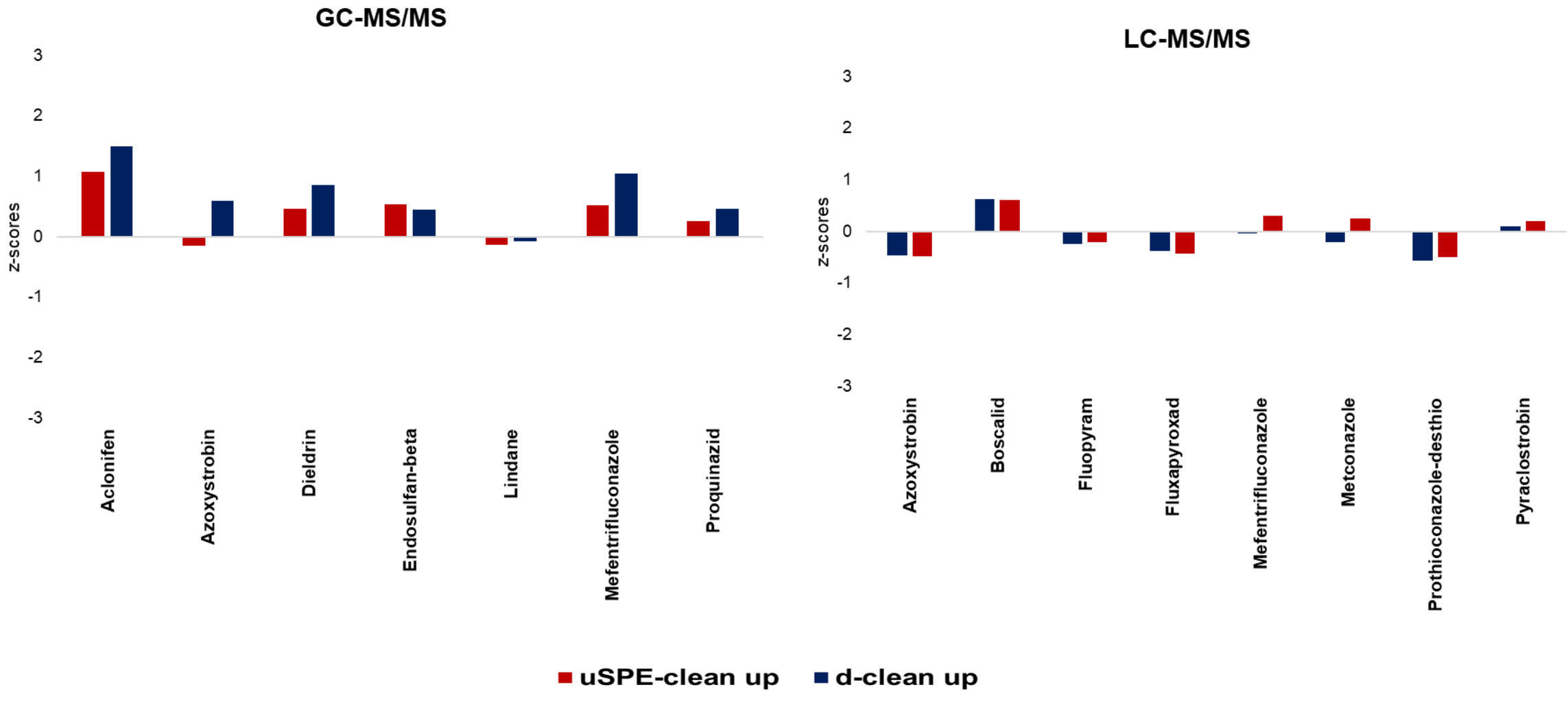
- Extending our validation scope in more difficult matrices using the µSPE clean up.
- **Transferring the knowledge.**
- Finalizing the automatic calibration curve.

Automated µSPE Extract Clean-up for pesticides in cereals



Automated µSPE Extract Clean-up for pesticides in cereals

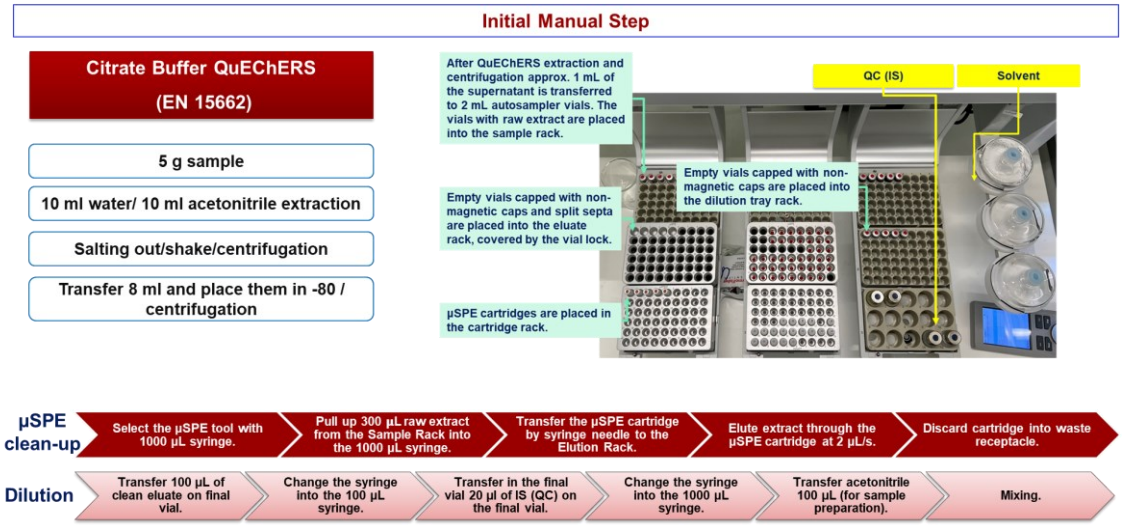
EURL CF-16 barley



Automated µSPE Extract Clean-up for pesticides in cereals

Hands on training I

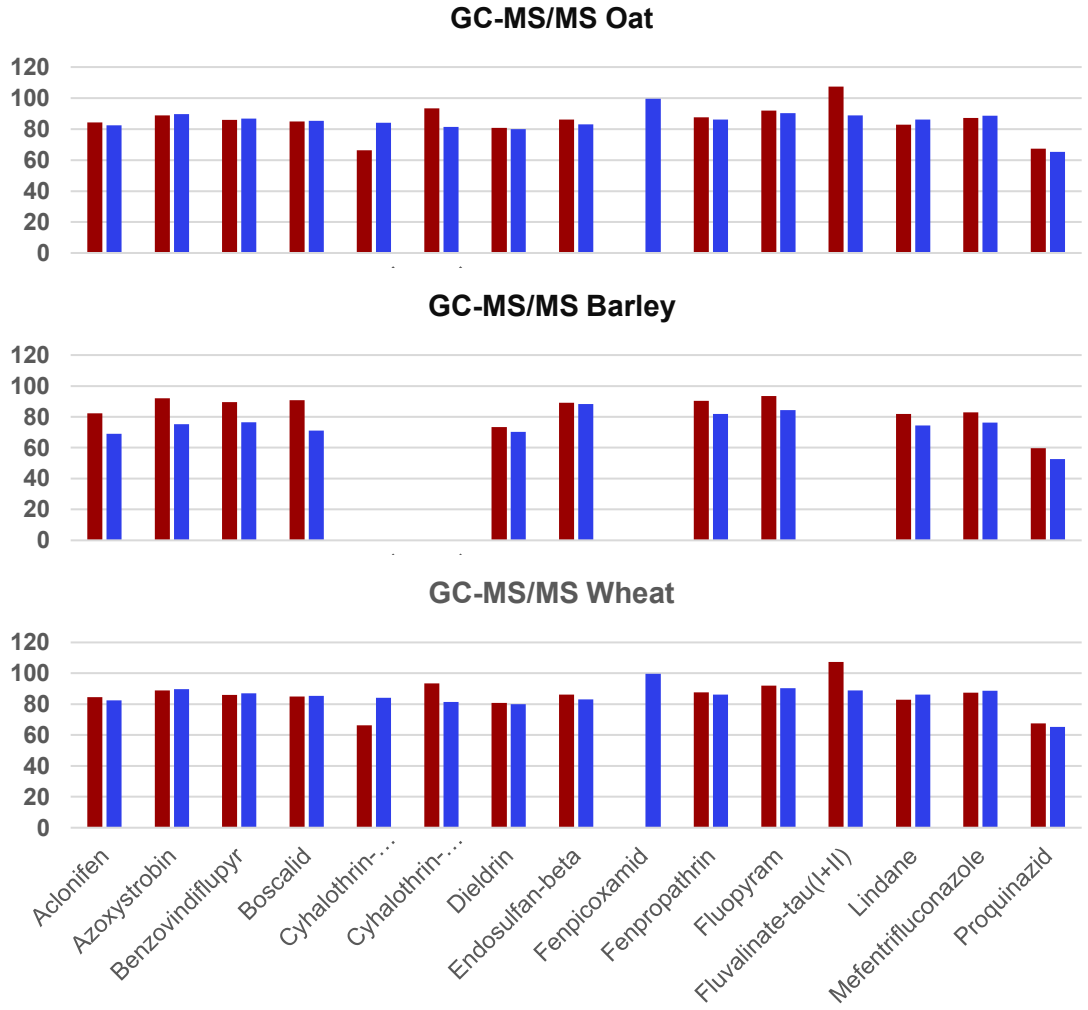
- Barley, group DTU spike 0.01 (*5) and 0.02 *(5) mg/kg
- Wheat, group 1 spike 0.01 (*5) and 0.02 (*5) mg/kg
- Oat, group 2 spike 0.01 (*5) and 0.02 (*5) mg/kg



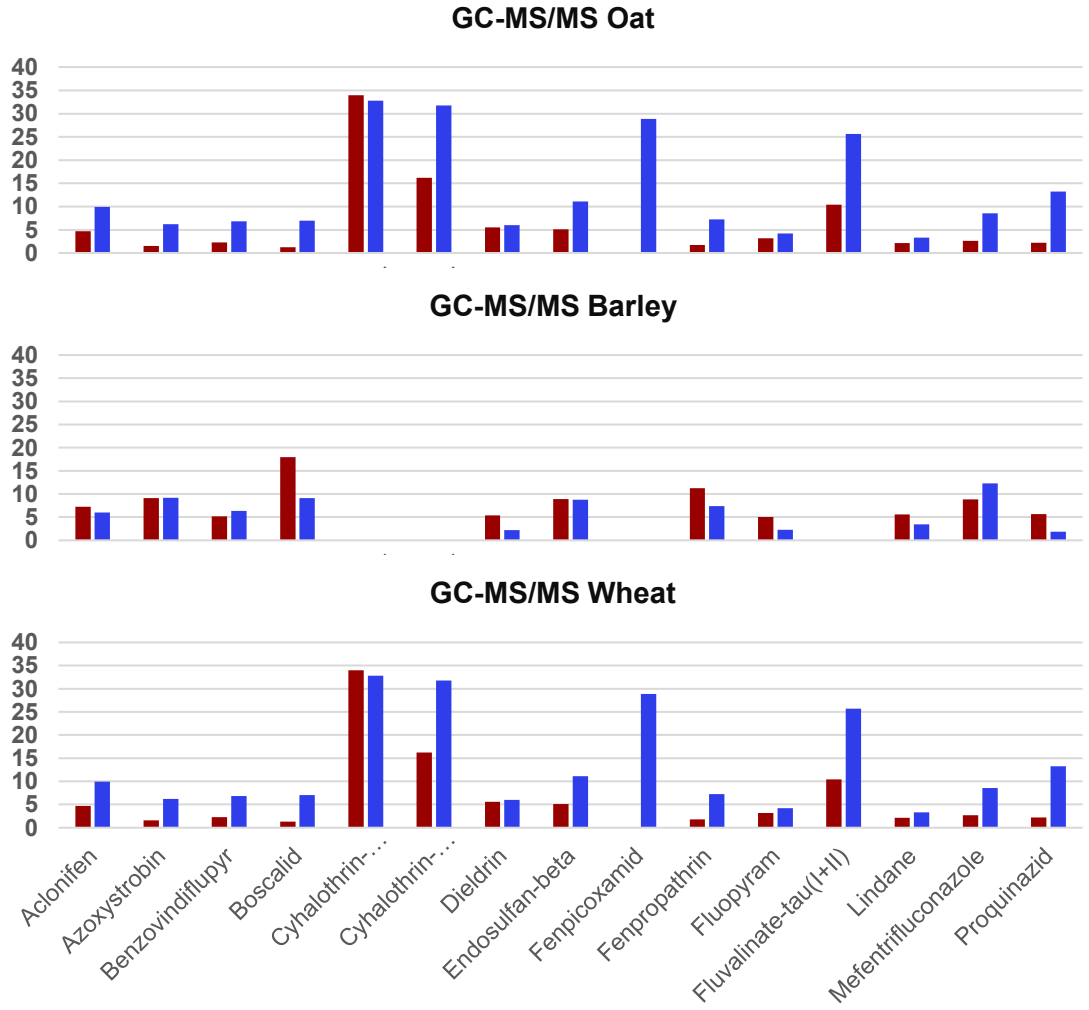
Automated µSPE Extract Clean-up for pesticides in cereals

■ 0.01 mg/kg ■ 0.02 mg/kg

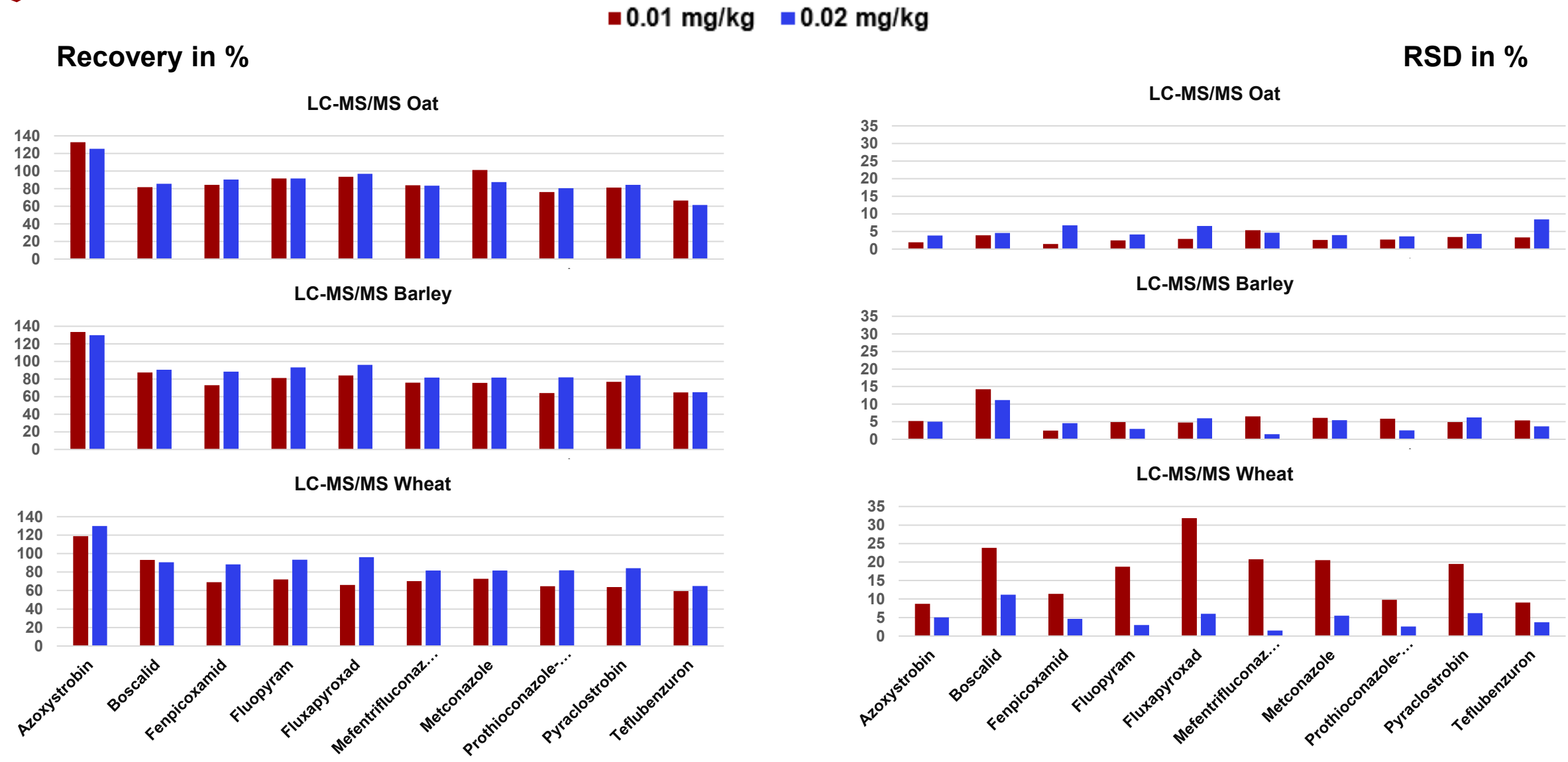
Recovery in %



RSD in %



Automated µSPE Extract Clean-up for pesticides in cereals

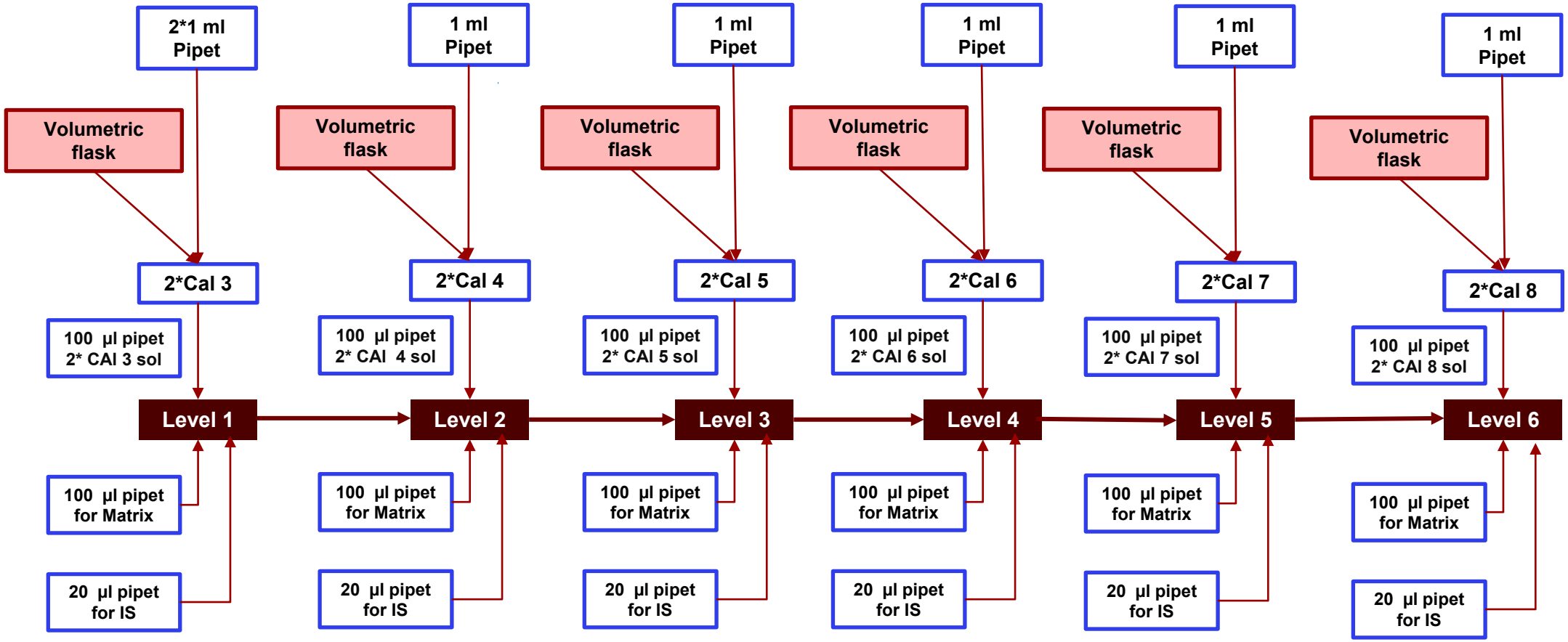


2021 – Future work

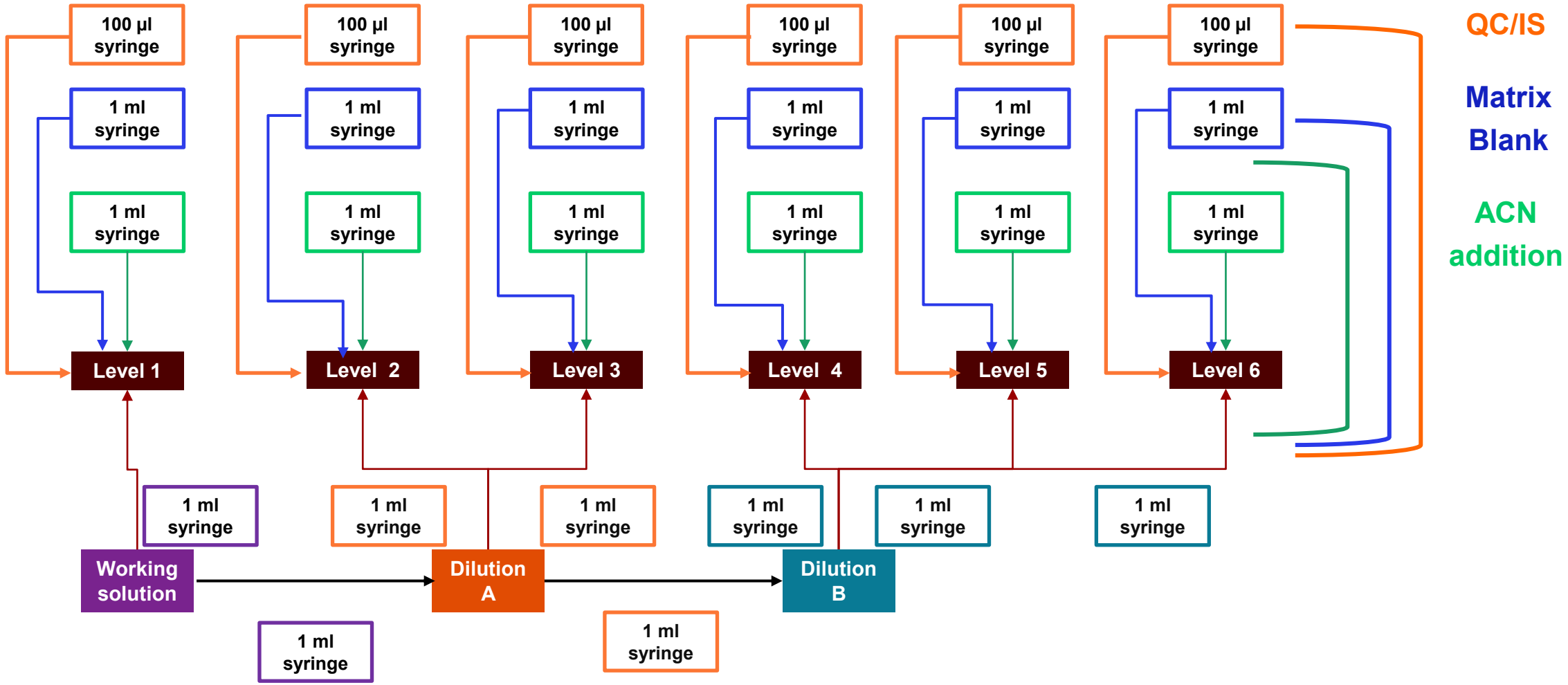
- Extending our validation scope in more difficult matrices using the µSPE clean up.
- Transferring the knowledge.
- **Finalizing the automatic calibration curve.**

Uncertainty contribution during manually preparation of the calibration curve

Working solution



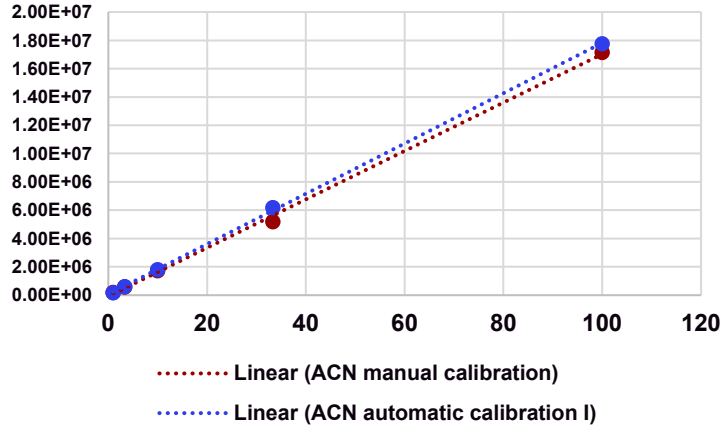
Uncertainty contribution during automatic preparation of the calibration curve



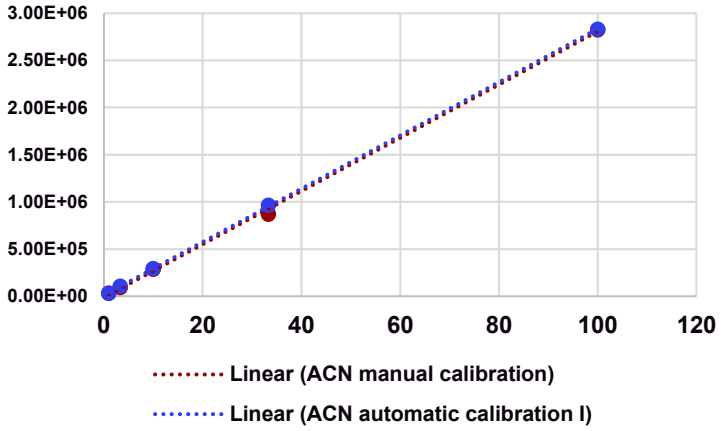
Automatic Preparation of Calibration Curve

Comparison between manually and automatic prepared calibration curves

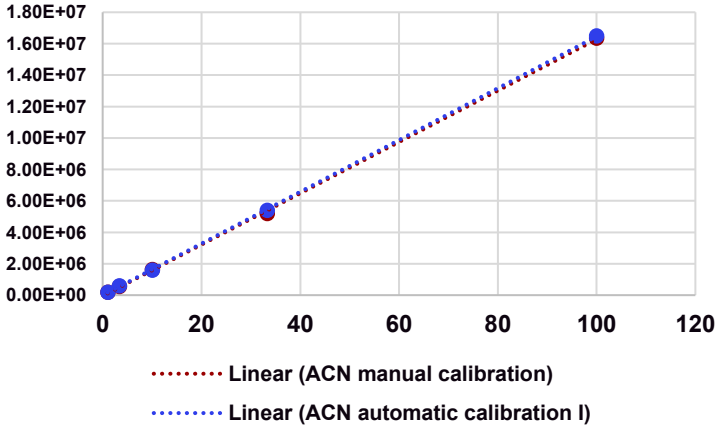
Azoxystrobin



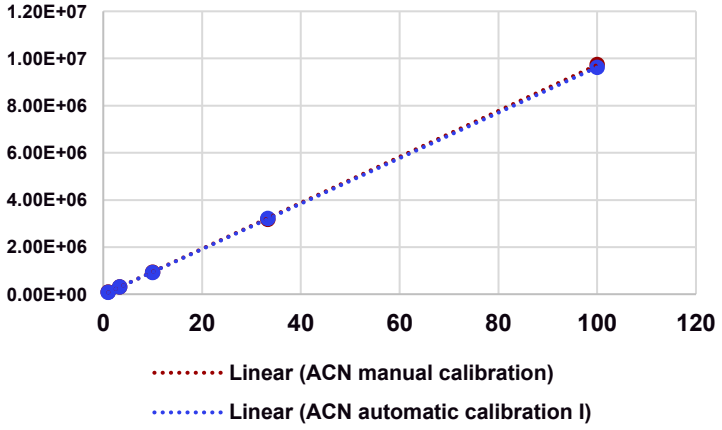
Chlorpyrifos



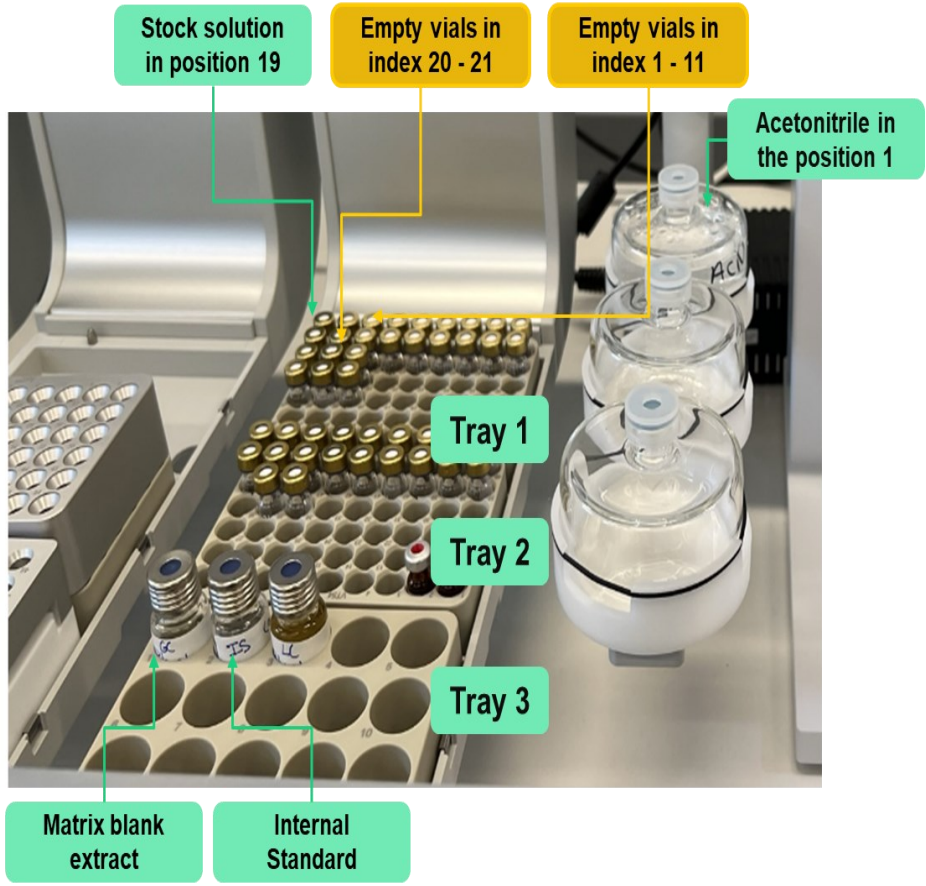
Dimethoate



Methamidophos



Stand Alone Multi-purpose Autosampler



Fully automatic matrix matched calibration curve in blank matrix extract

Preparation of Calibration Curve

Dilution from 10 to 3000-fold were prepared by using the automatic dilution workflow starting from working solution mix (2 mg/mL-manually prepared).

Solutions with concentration 0.2, 0.06, 0.02, 0.066, 0.002 and 0.0006 mg/L, were prepared in acetonitrile (workflow design by DTU/CTC on Chromeleon software).

µSPE Clean-up

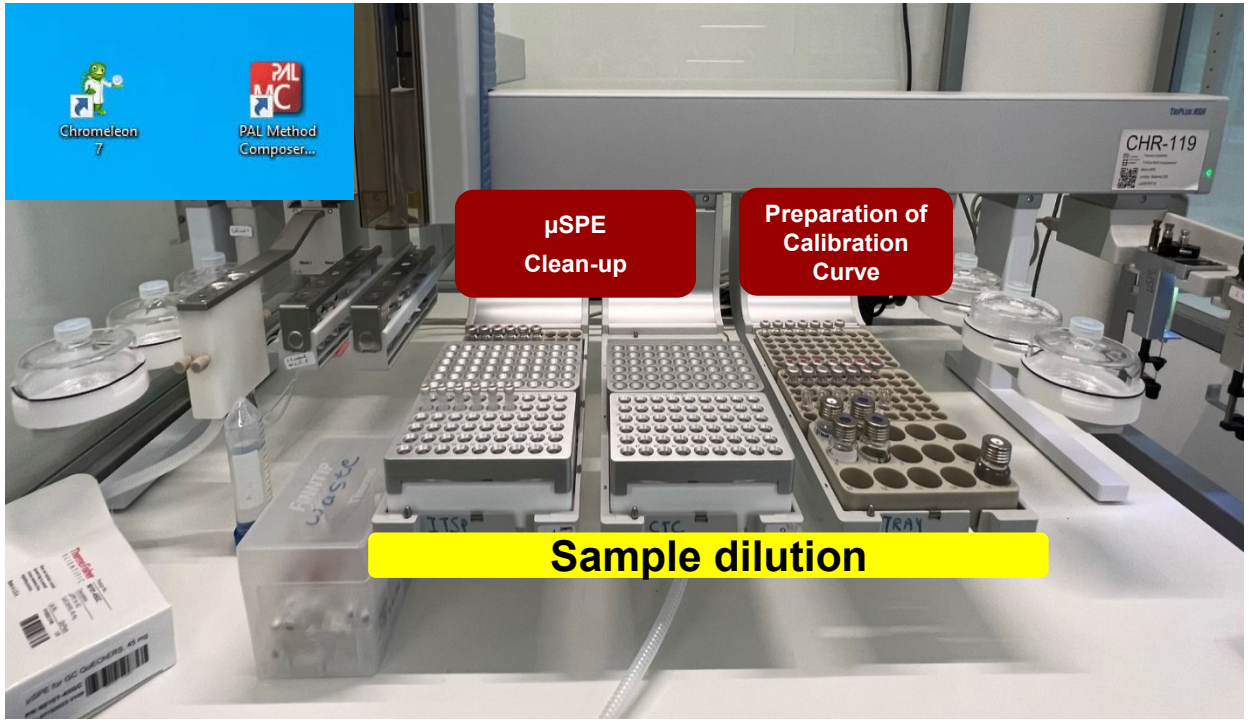
Blank extract was cleaned through the µSPE cartridge (workflow design by DTU/CTC on Chromeleon software).

Sample dilution

100 µl from each calibration solution was automatically diluted with 100 µl of matrix extracted previously clean through the ITSP.

Final concentrations were 0.1, 0.033, 0.01, 0.0033, 0.001 and 0.0003 mg/L (workflow was designed by DTU user using the PAL method composer).

Stand Alone Multi-purpose Autosampler

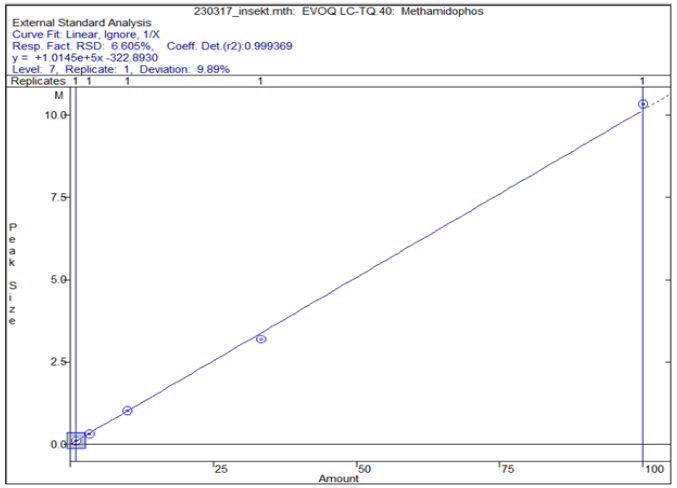


Fully automatic matrix matched calibration curve in blank matrix extract

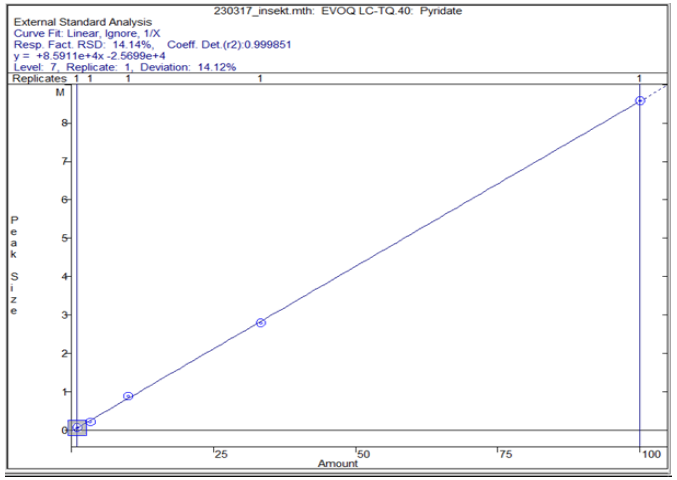
LC

GC

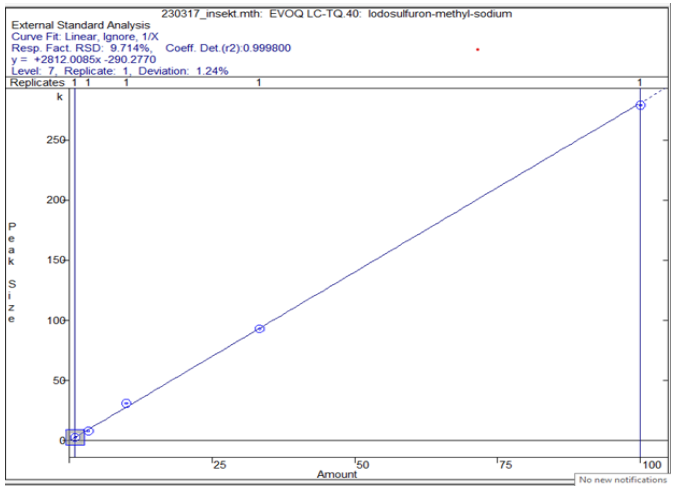
Methamidophos



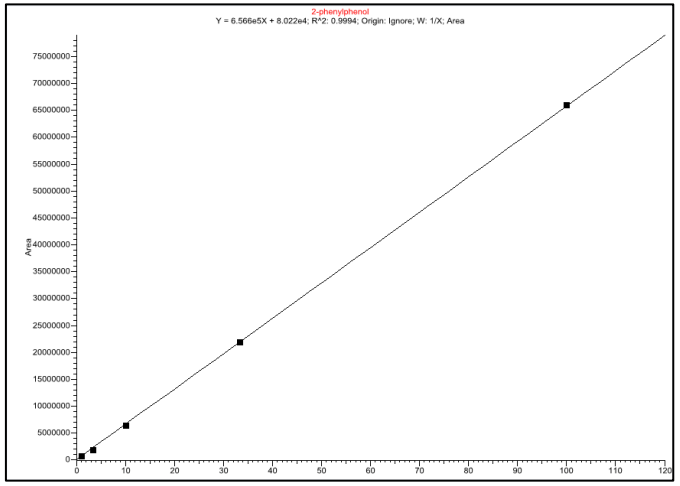
Pyridate



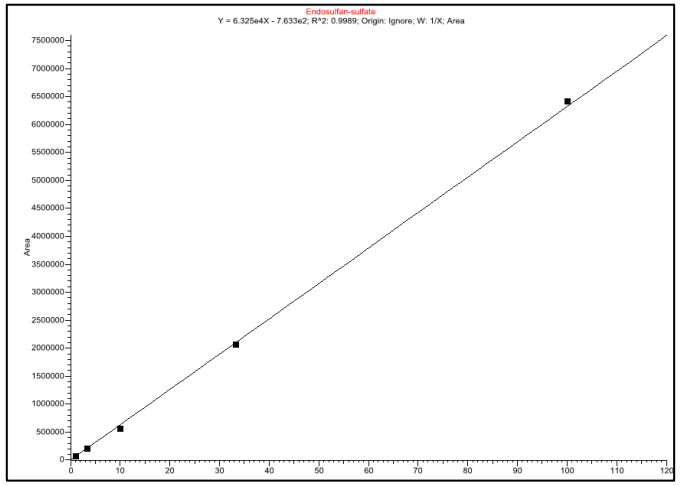
Iodosulfuron-methyl-sodium



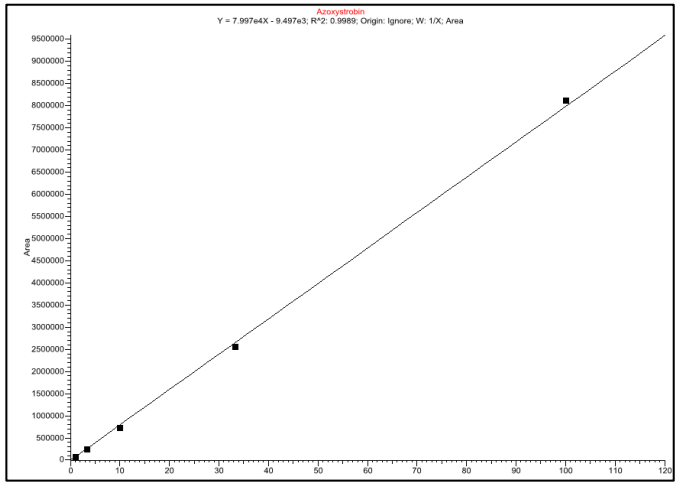
2-phenylphenol



Endosulfan-sulfate



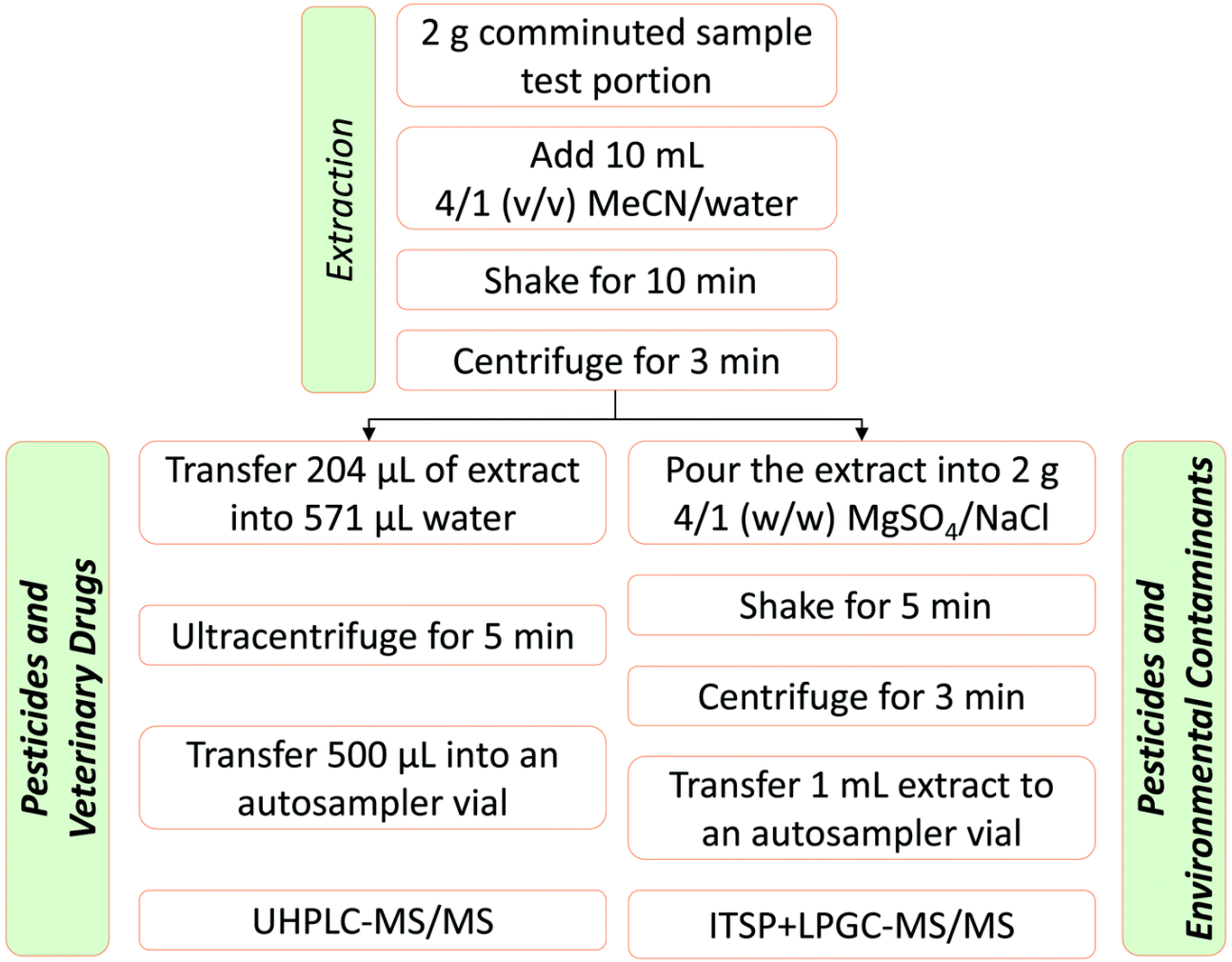
Azoxystrobin



Determination of pesticides in insects using automatic sample preparation

QuEChERSER method

"Quick, Easy, Cheap, Effective, Rugged, Safe, Efficient and Robust".



Ninga et al., 2022- Analytical Methods, 14 (2022), pp. 2761-2770, 10.1039/d2ay00713d

ITSP
Instrument Top Sample Preparation

Source: Lehotay, S. J.
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center; 600 East Mermaid Lane; Wyndmoor, PA 19038; USA

Flowchart of QuEChERSER method for determination of pesticides in insects

2 g of test portion

Add 10 ml (8/2 MeCN/H_2O)

Shake 5 min at 1000 rpm.

Centrifuge 3 min 4500 rpm.

Pure the extract into:

2 g $MgSO_4$/ 0.5g NaCl/ 0.5 g Na_3 citrate dihydrate and 0.25 g Na_2H citrate sesquihydrate.

Shake 5 min at 1000 rpm.

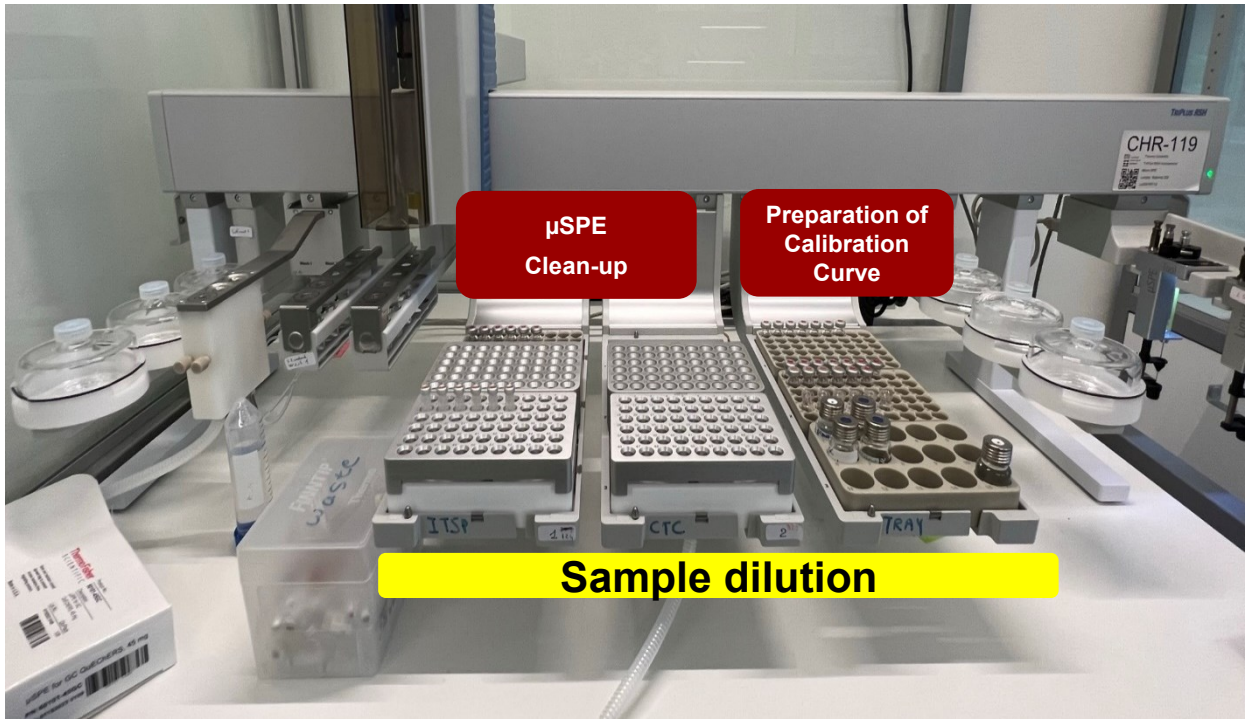
Centrifuge 3 min at 4500 rpm.

Transfer 1 mL of upper layer on an autosampler vial

Stand-Alone Multi-purpose Autosampler

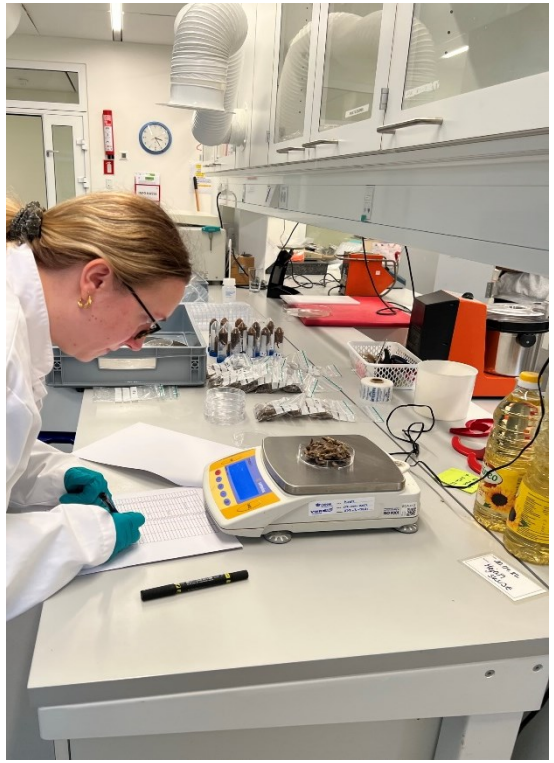
GC-MS/MS and/or LC-MS/MS

Stand Alone Multi-purpose Autosampler



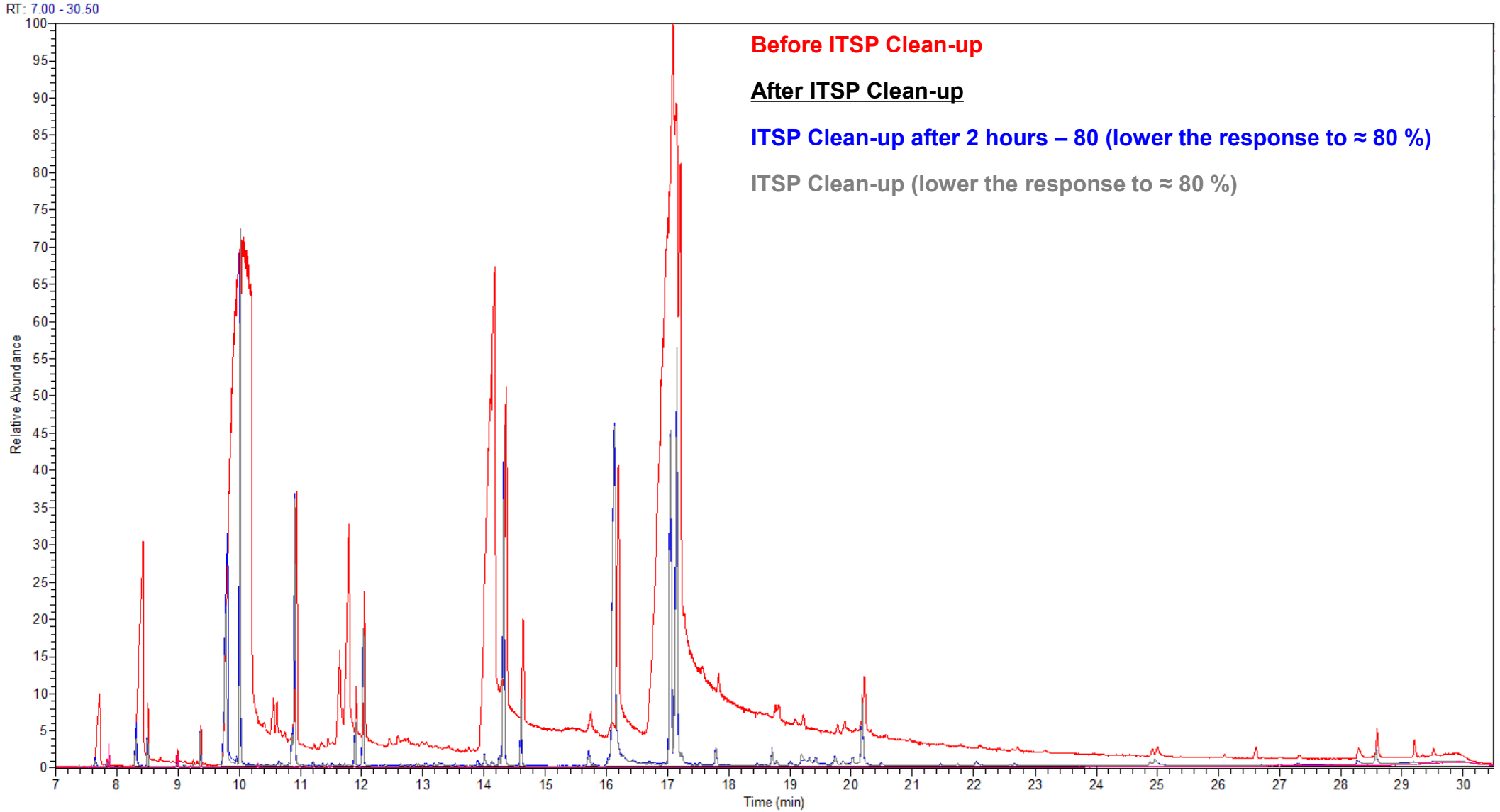
National Food Institute, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark

Determination of pesticides in insects - sample preparation

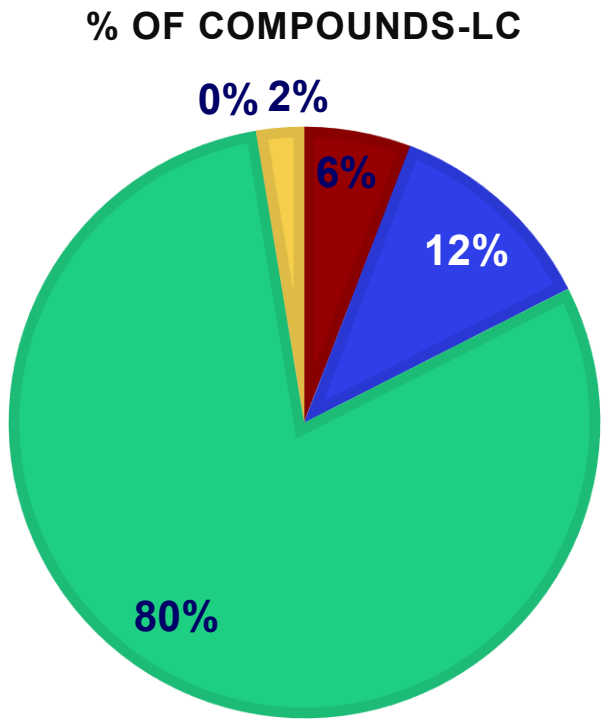


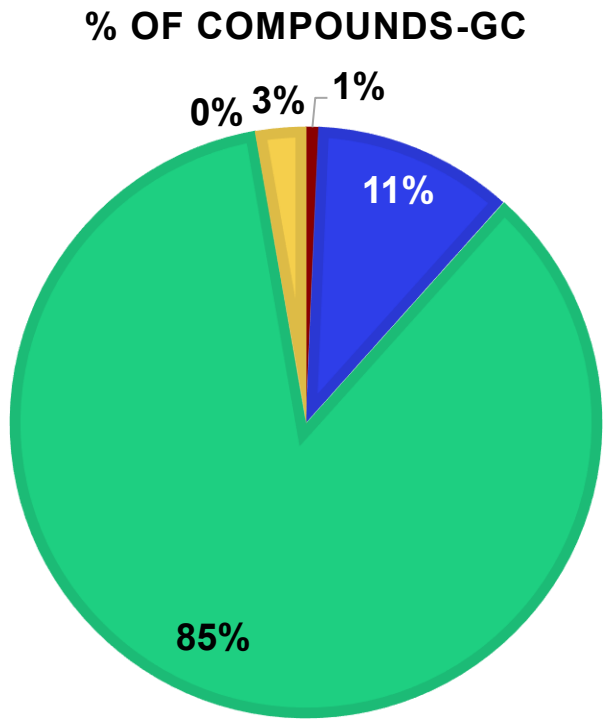
Freeze dry samples were homogenized in a blender in presence of dry ice.

Full Scan GC-MS of insect larvae



Recovery of spiked extract (50 µg/kg) before the µSPE clean up





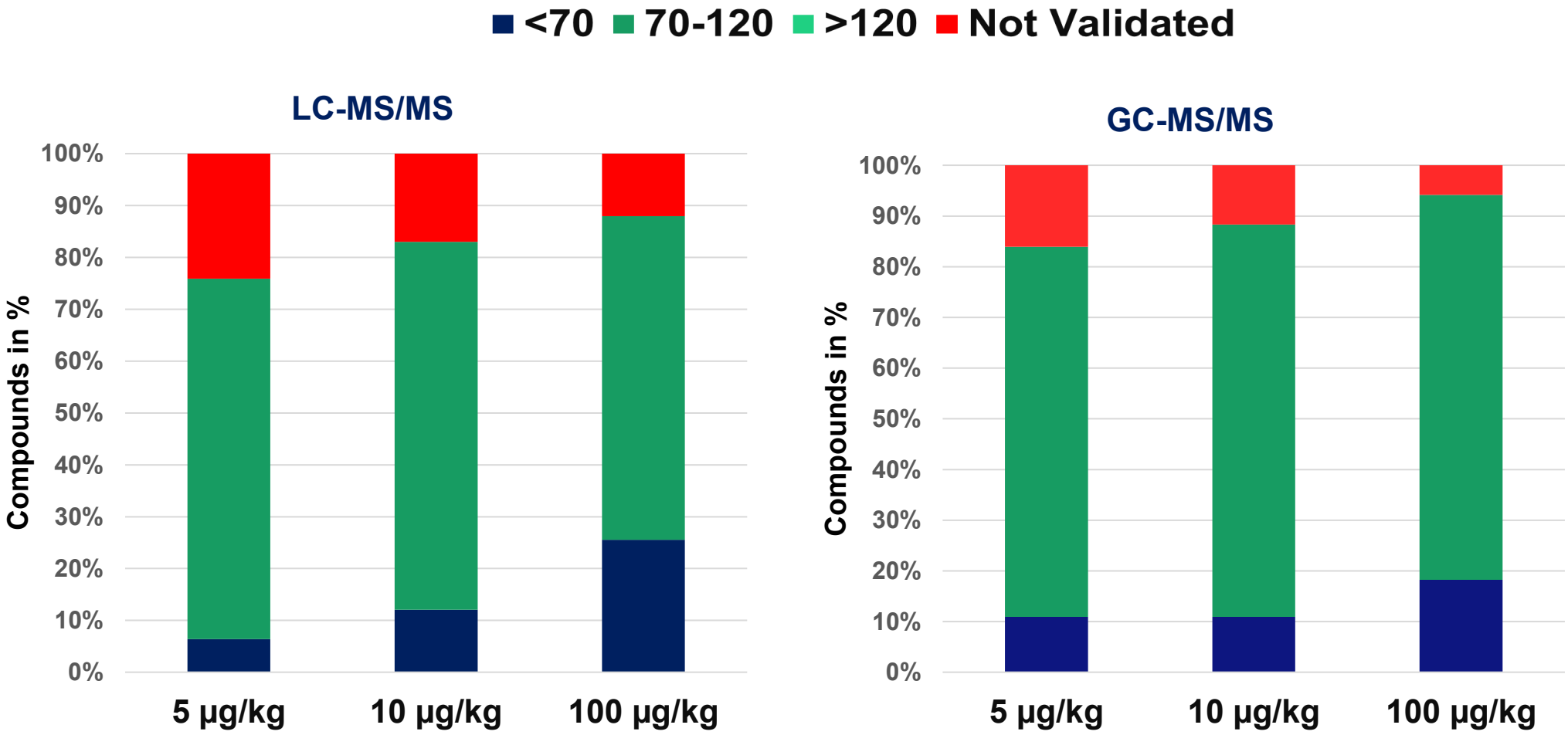
Compounds with low recovery (<40 %) -LC ; Bromoxynil, Fenpropidin, Iodosulfuron-methyl-sodium, Metsulfuron-methyl, Propamocarb, Prosulfuron, Spinosad_A/D, Spiroxamine.

Haloxyfop did not pass through the µSPE column.

Determination of pesticides in insects - Validation design

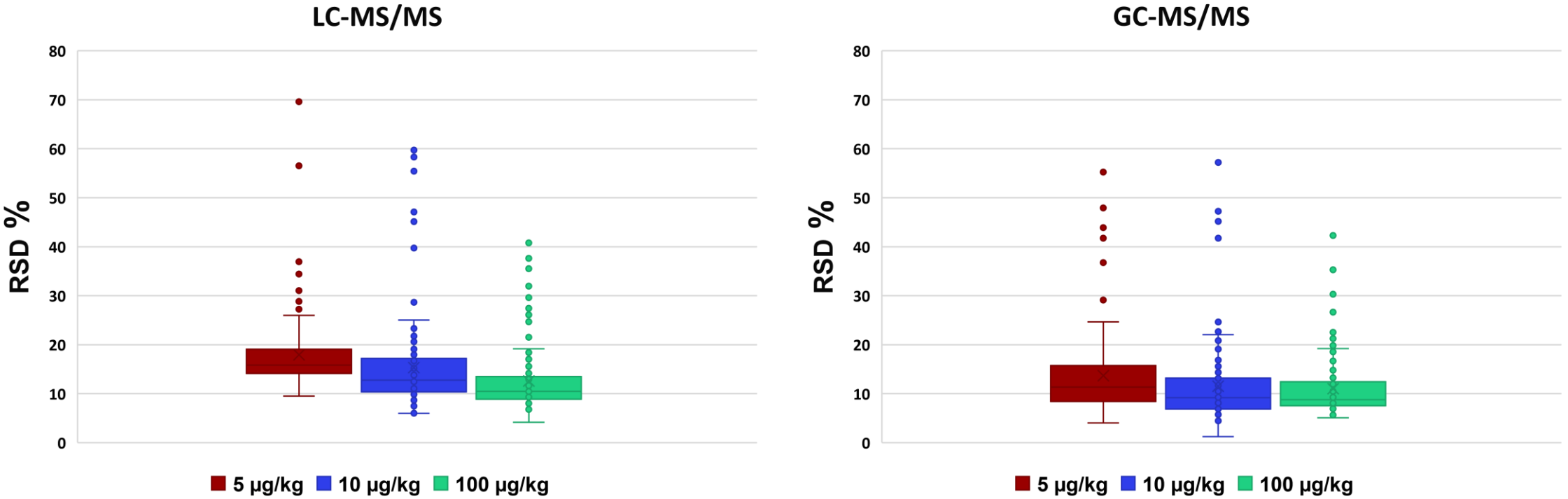
Day	Quantitative analysis 1	BSF larvea	MO larvae	Tech	Date
1	Initial full validation	6 replicates * 3 level		1	January – April 2023
2	Initial full validation		6 replicates * 3 level	2	
3	On going validation	2 replicates * 3 level		1	
4	On going validation		2 replicates * 3 level	2	
5	On going validation	2 replicates * 3 level		3	
6	On going validation		2 replicates * 3 level	3	
7	On going validation	2 replicates * 3 level		3	
8	On going validation		2 replicates * 3 level	1	

Determination of pesticides in insects - Recovery
Still on going



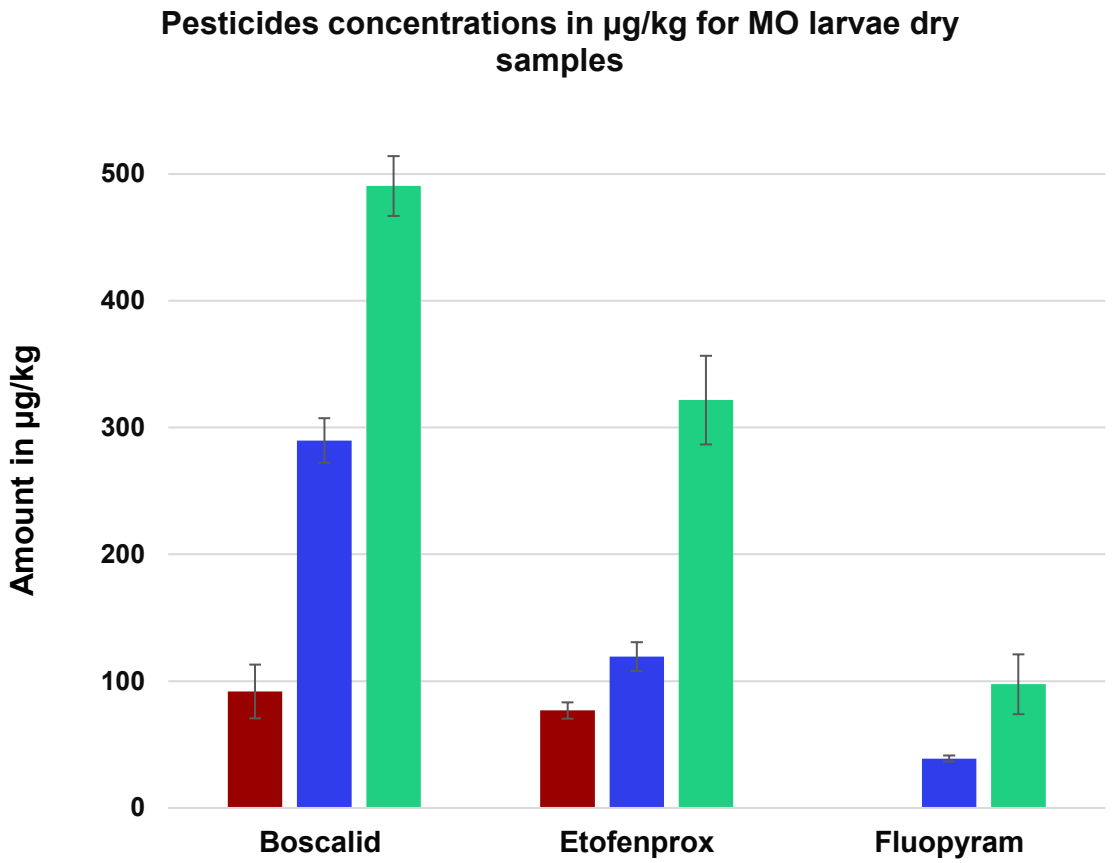
Distribution of recoveries (at three spiking levels), for BSF and MO larvae.

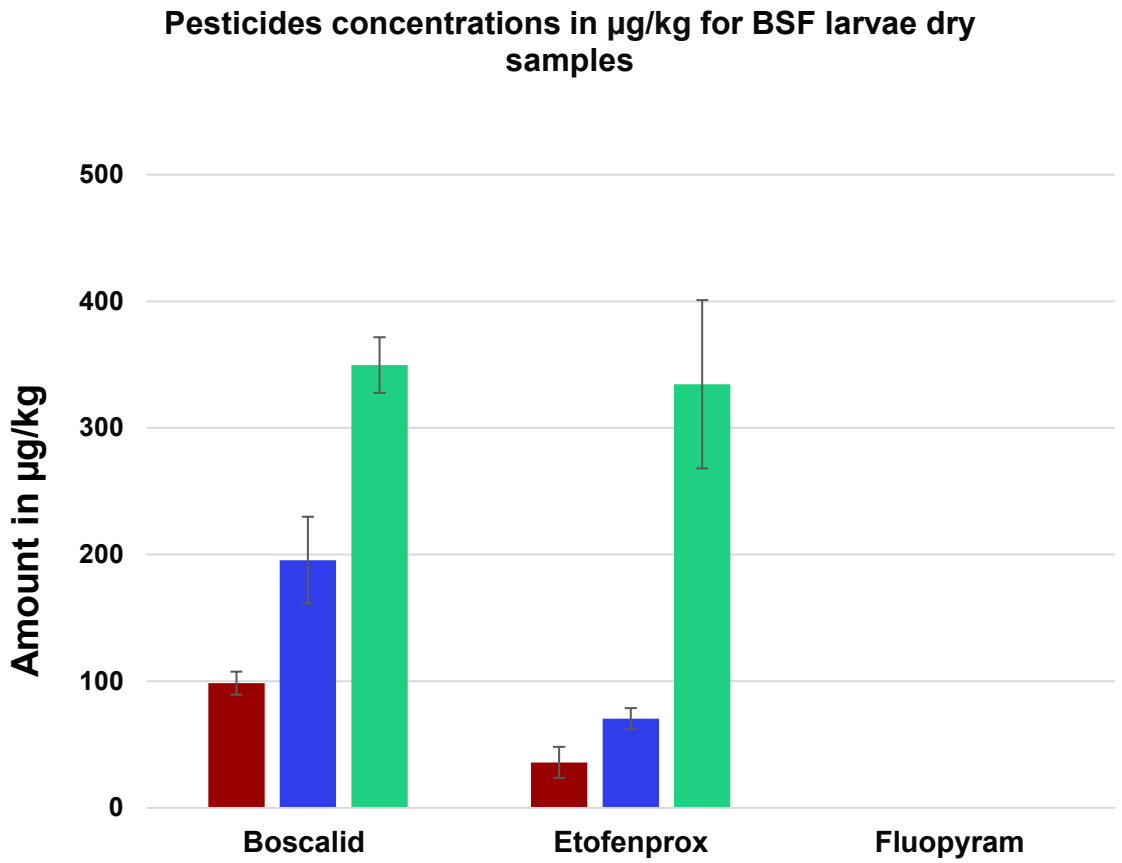
Determination of pesticides in insects - RSD



RSD % (at three spiking levels), for both BSF and MO larvae.

Determination of pesticides in insects - Samples





Acknowledges

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Arvid Fromberg

Nick Kjærgaard Dahlberg

Ban M. Kadhum

Thank you for your attention!

edni@food.dtu.dk