NEW ADVANCES IN THE ANALYSIS OF MRM COMPOUNDS

DIFFERENT EXTRACTION APPROACHES FOR HIGH PROTEIN CONTENT PULSES

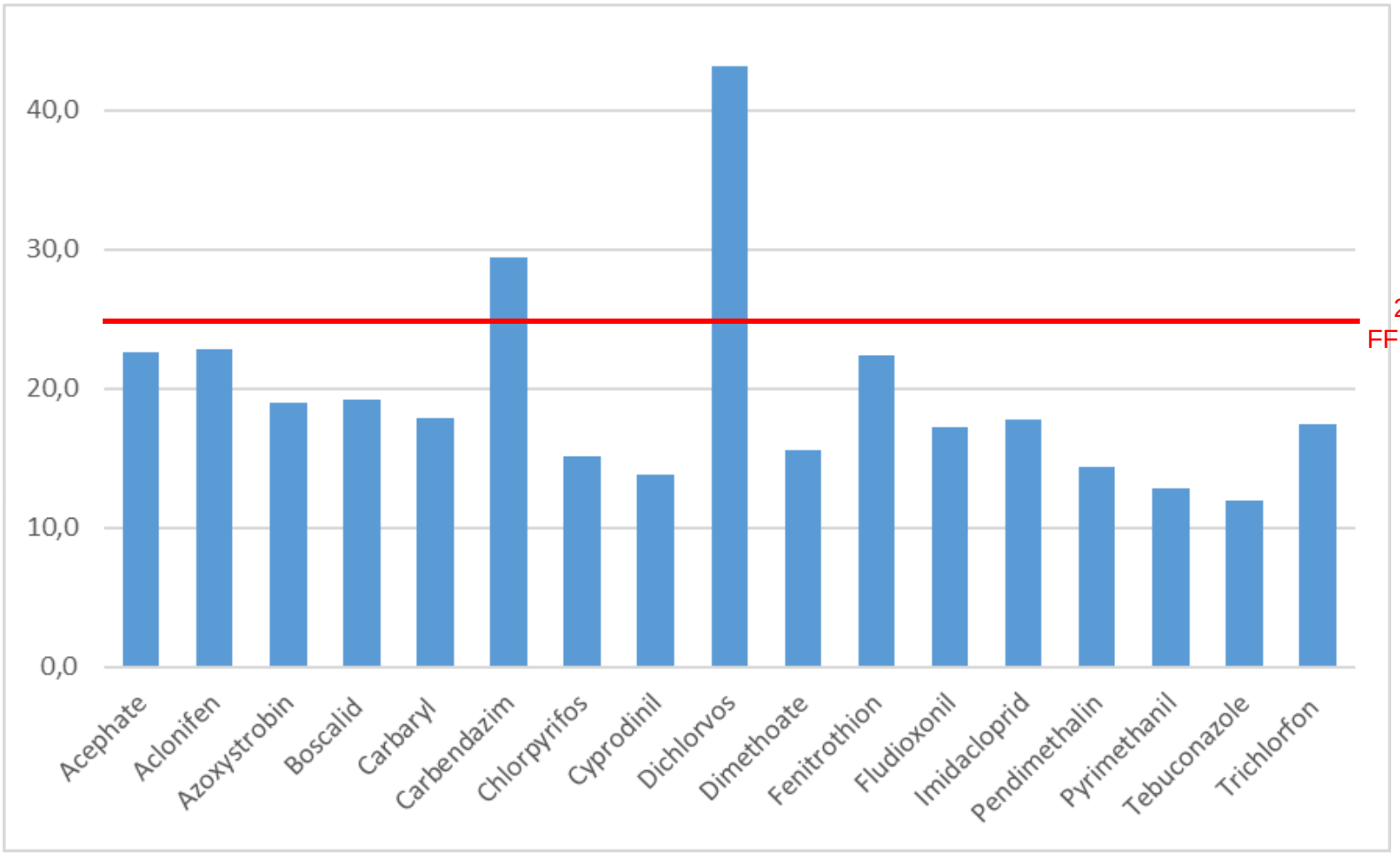
JOSÉ ANTONIO MARTÍNEZ MARTÍNEZ

EUPT-FV-SC05

European Proficiency Test FV-SC05

White dried beans

Dispersion of Results



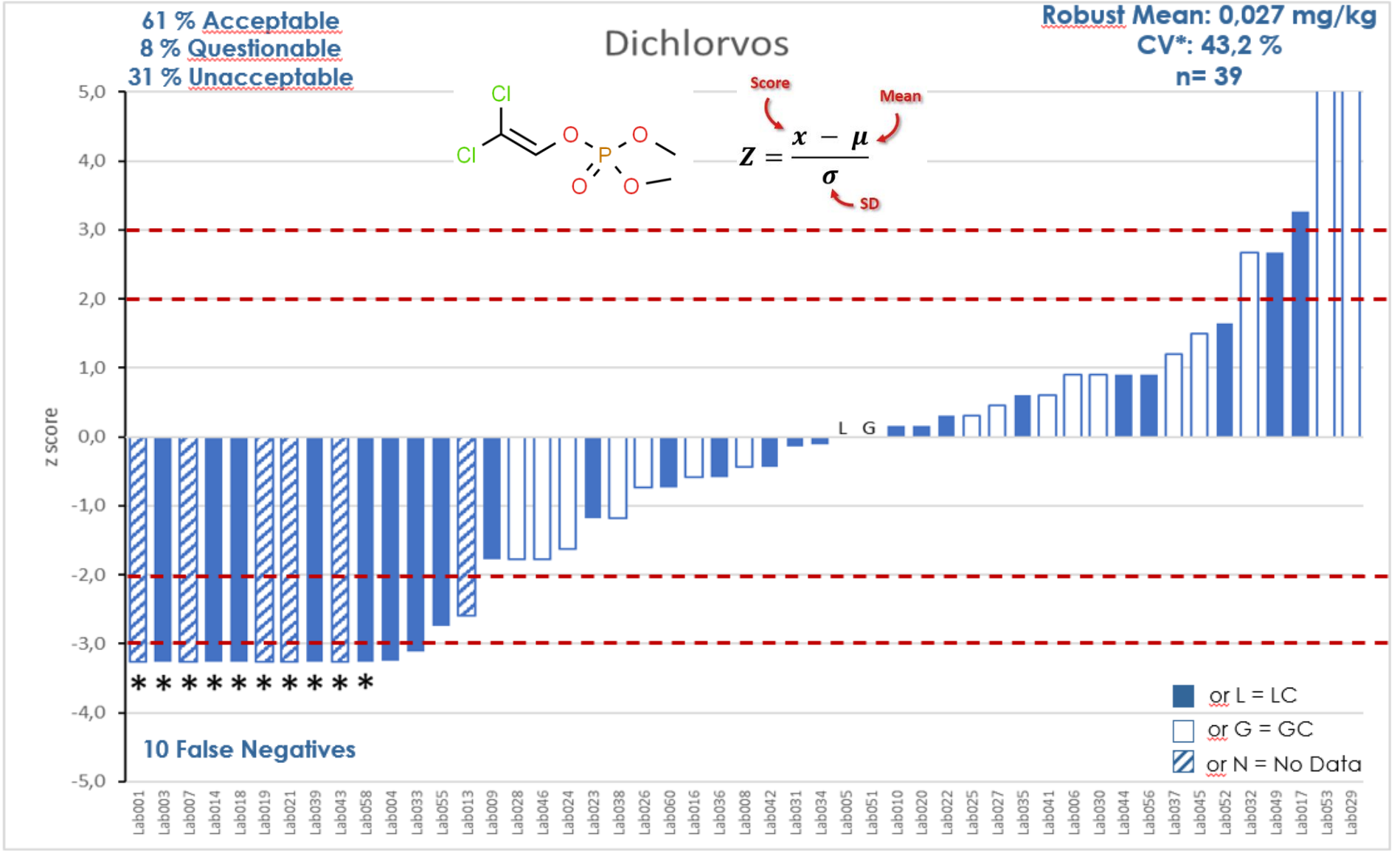
Different Extraction Approaches For High Protein Content Pulses

	No. of Reported Results	No. of False Negative Results	No. of Not Analysed Results	Percentage of Labs Reporting Results (out of 56)
Acephate	50	2	4	89
Aclonifen	38	0	18	68
Azoxystrobin	53	2	1	95
Boscalid	54	0	2	96
Carbaryl	53	0	3	95
Carbendazim	52	0	4	93
Chlorpyrifos	55	1	0	98
Cyprodinil	53	2	1	95
Dichlorvos	39	10	7	70
Dimethoate	54	1	1	96
Fenitrothion	53	0	3	95
Fludioxonil	52	2	2	93
Imidacloprid	53	0	3	95
Pendimethalin	54	1	1	96
Pyrimethanil	55	0	1	98
Tebuconazole	54	1	1	96
Trichlorfon	44	3	9	79

Different Extraction Approaches For High Protein Content Pulses

	Robust Mean (mg/kg)	% Acceptable z scores	% Questionable z scores	% Unacceptable z scores
Acephate	0,072	88	6	6
Aclonifen	0,039	97	0	3
Azoxystrobin	0,036	95	0	5
Boscalid	0,058	96	2	2
Carbaryl	0,049	96	4	0
Carbendazim	0,064	88	6	6
Chlorpyrifos	0,076	93	0	7
Cyprodinil	0,051	95	0	5
Dichlorvos	0,027	61	8	31
Dimethoate	0,033	96	2	2
Fenitrothion	0,060	92	6	2
Fludioxonil	0,078	96	2	2
Imidacloprid	0,081	98	0	2
Pendimethalin	0,034	94	0	6
Pyrimethanil	0,035	96	4	0
Tebuconazole	0,044	96	2	2
Trichlorfon	0,068	95	5	0

Different Extraction Approaches For High Protein Content Pulses



Different Extraction Approaches For High Protein Content Pulses

- Sante Document recommends sample hydration prior to extraction
- Sample hydration increases extraction of polar compounds, but may hinder the extraction of certain apolar compounds
- Coextraction of other matrix components can be the source of matrix interferences in the analysis of target analytes
- In this case the high content of proteins with amino groups can interact with organochlorine compounds

To hydrate the sample or not to hydrate the sample?

4 method of Extraction

White bean and pinto bean

- H_2O + SDS
- 30 min shaking
- H_2O
- AcN

- H2O: Evaluating the effect of hydration
- AcN: Evaluating the effect of non-hydration
- 30 min shaking: We evaluate the effect of longer agitation time
- H2O+SDS: We evaluate hydration, while seeking to denature the proteins present in the matrix

Extraction SDS

H_2O + SDS

Sample

5 ± 0.05 g

10 ml H2O + 0.25 g SDS

shake manually 3s aprox.

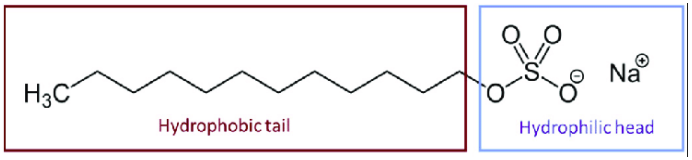
10 ml ACN

agitator 6min

shake manually 3s aprox.

6,5 g salts
(Mix de 1g $NaCl_2$, 4g $MgSO_4$, 1g $C_6H_5Na_3O_7$ * 2 H_2O, 0,5g $Na_2HC_6H_5O_7$.1 1/2H_2O)

agitator 6min



Centrifuge
10 min
(3700 rpm)

8ml extract
in dry ice
20 min

Centrifuge
1 min
(3700 rpm)

Clean-up
5 ml in
125 mg PSA
+750 mg MgSO4

Vórtex 30s

Centrifuge
5 min
(3700 rpm)

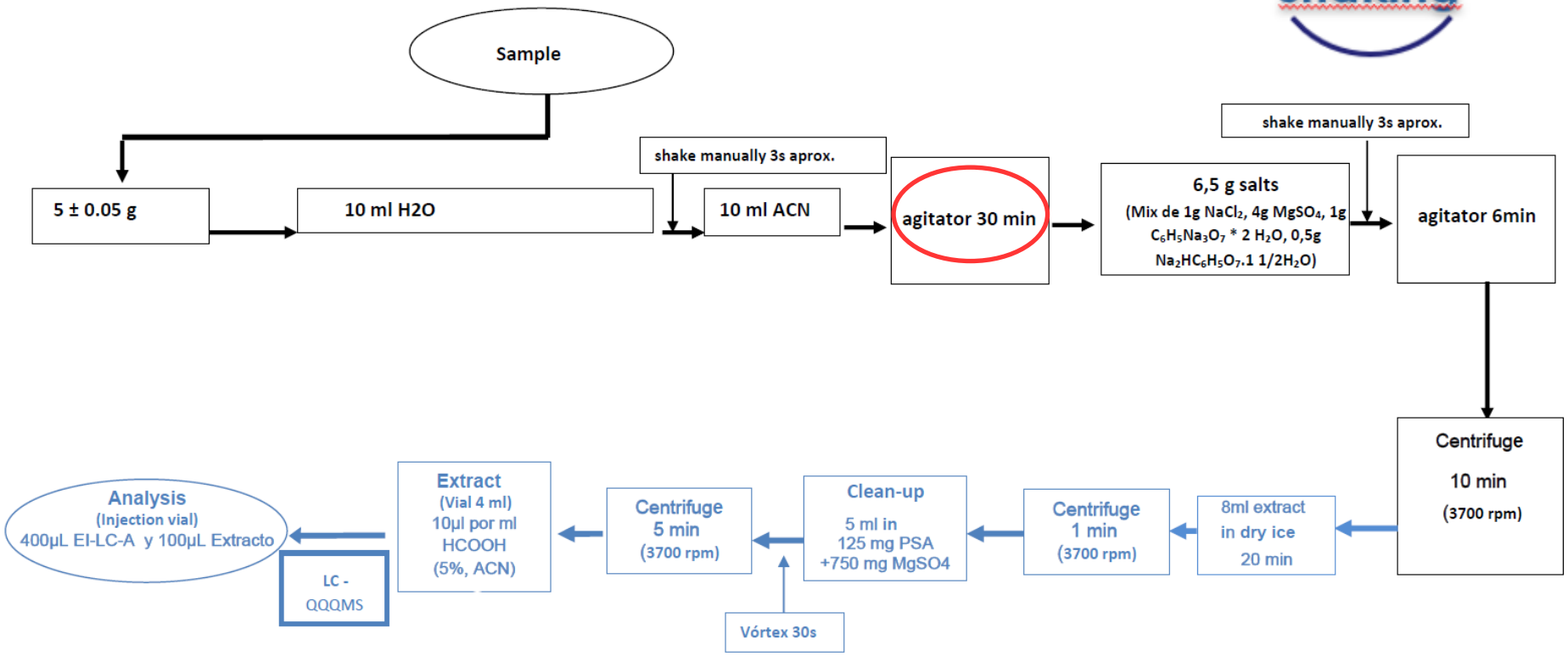
Extract
(Vial 4 ml)
10µl por ml
HCOOH
(5%, ACN)

LC -
QQQMS

Analysis
(Injection vial)
400µL EI-LC-A y 100µL Extracto

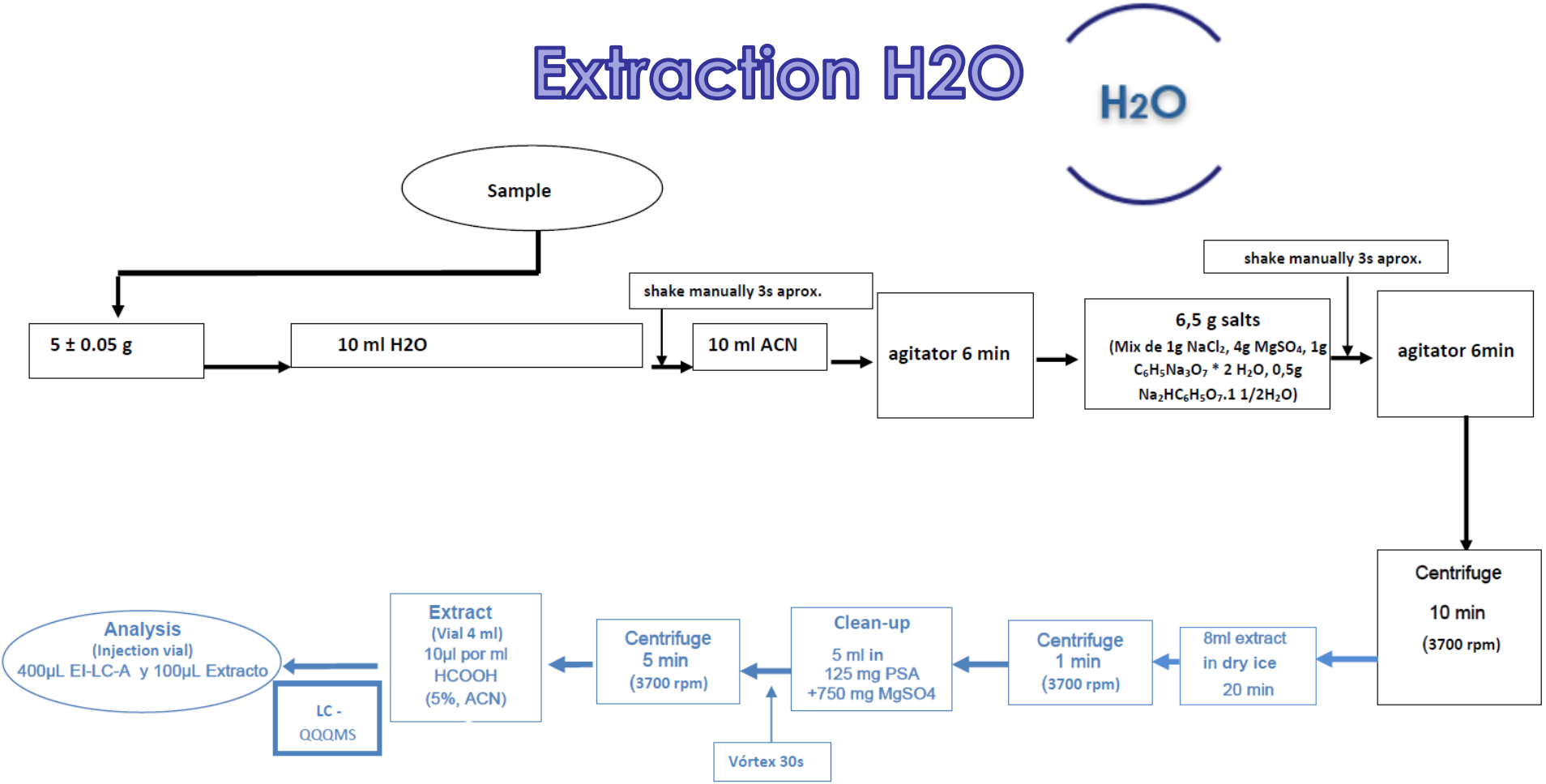
Preparation of calibration line in solvent (ppm)
Preparation of calibration line in matrix (ppm) LC: Evap. 100 µL extract blank y reconst. 100 µL point in solvent- 400 µL H2O (EI-LC-A)

Extraction 30 min. shaking



Preparation of calibration line in solvent (ppm)
Preparation of calibration line in matrix (ppm) LC: Evap. 100 µL extract blank y reconst. 100 µL point in solvent- 400 µL H2O (EI-LC-A)

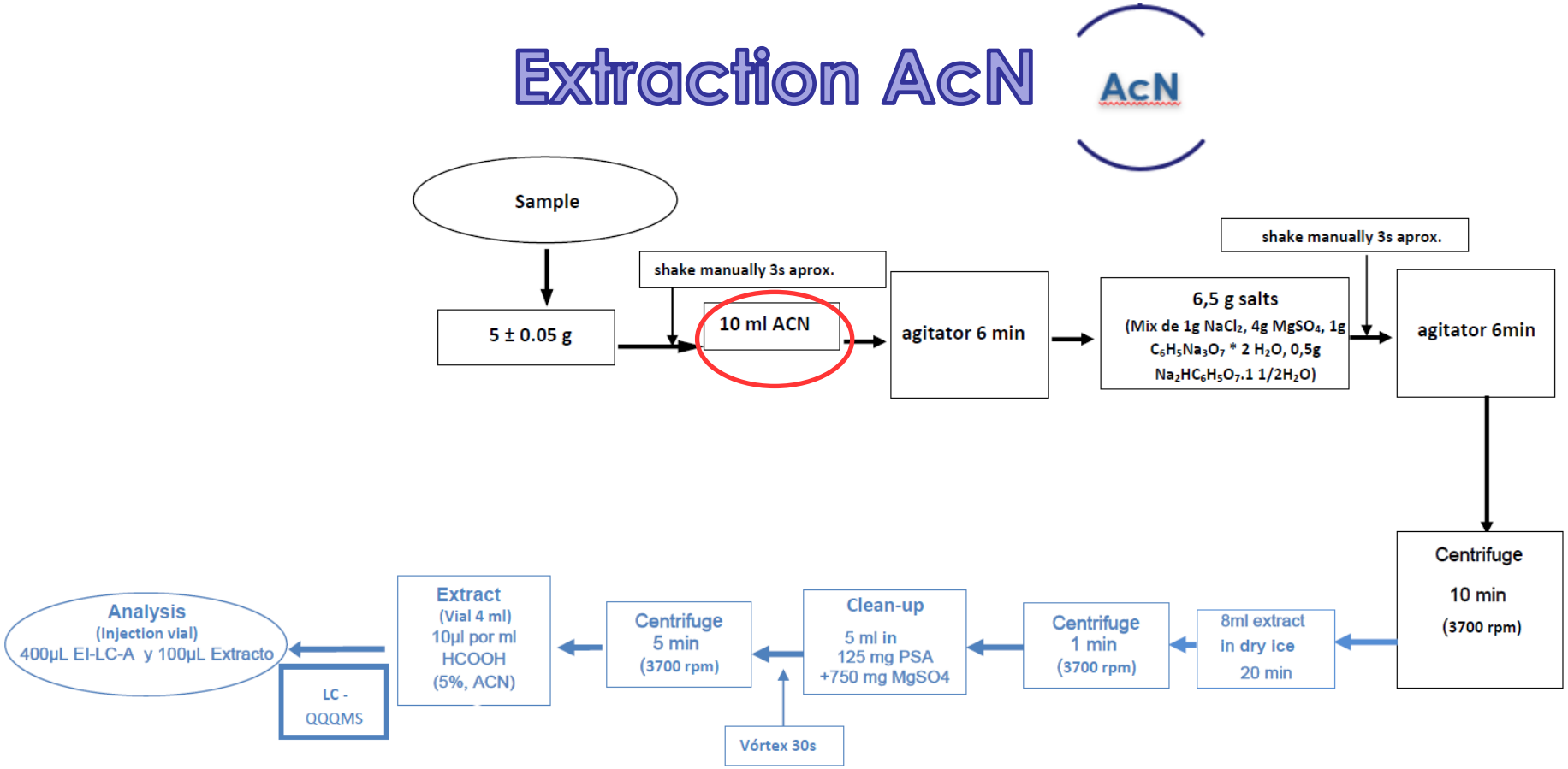
Extraction H2O



Preparation of calibration line in solvent (ppm)
Preparation of calibration line in matrix (ppm) LC: Evap. 100 µL extract blank y reconst. 100 µL point in solvent- 400 µL H2O (EI-LC-A)

Different Extraction Approaches For High Protein Content Pulses

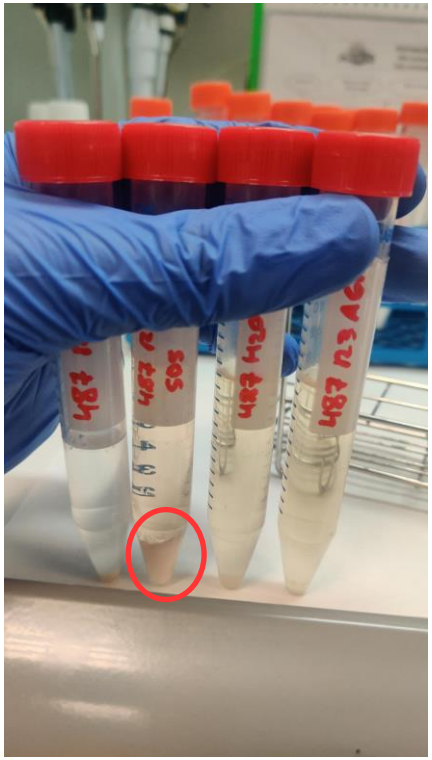
Extraction AcN



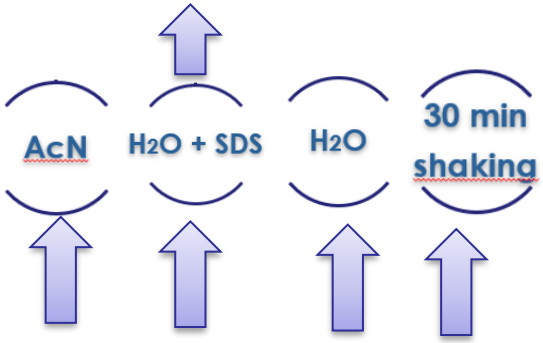
Preparation of calibration line in solvent (ppm)
Preparation of calibration line in matrix (ppm) LC: Evap. 100 µL extract blank y reconst. 100 µL point in solvent- 400 µL H2O (EI-LC-A)

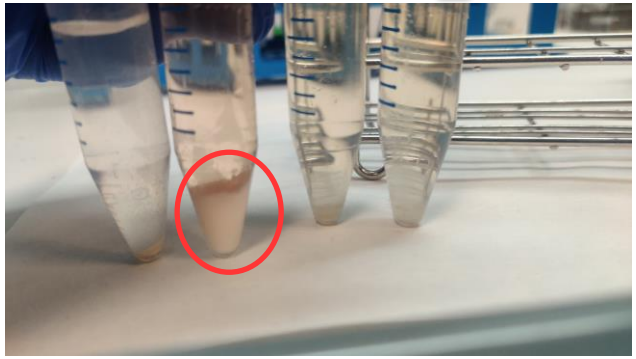
4 method of Extraction

photograph centrifugation after the freezing out stage



It can be observed that the protein precipitate is higher in the SDS method.





Analysis Samples

UHPLC (Thermo ScientificTM TranscendTM DUO LX-2 LC)

- Column: Accucore C18 2.1x100 mm and 2.6 µm particle size (Thermo Scientific™)
- Mobile phase A: Water (0.1 % formic acid, 5 mM ammonium formate, 2 % MeOH)
- Mobile phase B: Methanol (0.1 % formic acid, 5 mM ammonium formate, 2 % water)
- Column temperature: 30 °C
- Flow rate: 0.35 ml/min
- Injection volume: 2.5 µL
- Autosampler temperature: 10 °C

Mobile phase gradient for pesticides analysis:

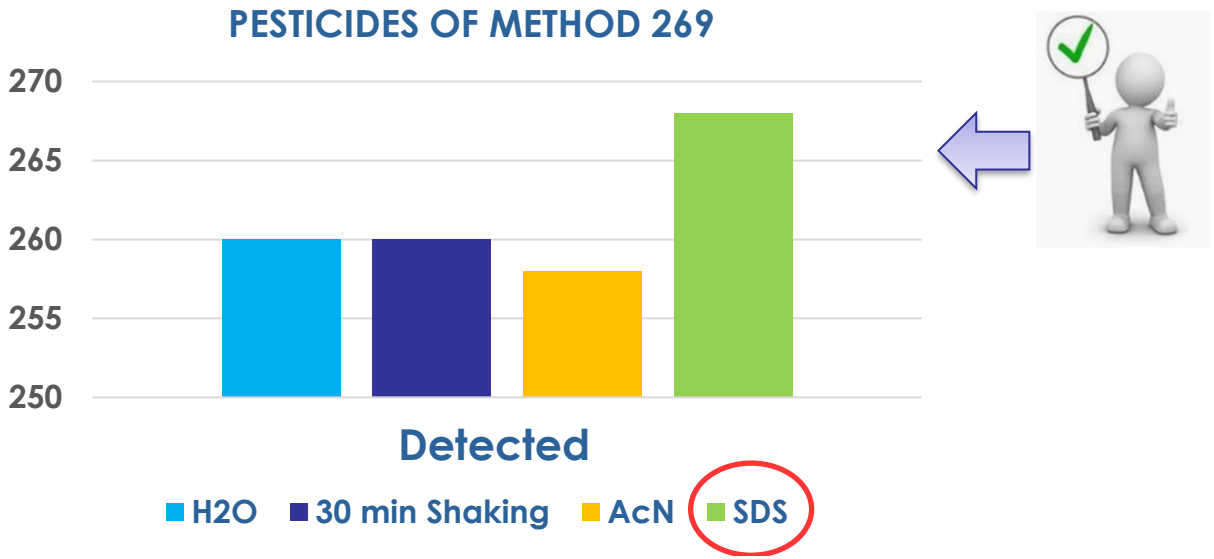
Time [min]	Mobile phase A
0	100 %
1	100 %
2	70 %
3	50 %
11	0 %
14	0 %
14.1	100 %
17	100 %
Data window [min]	1.1-11.55

Triple quadrupole system (Transcend DUO LX-2. A TSQ Altis, Thermo Scientific)

Ion source: Opta Max NG

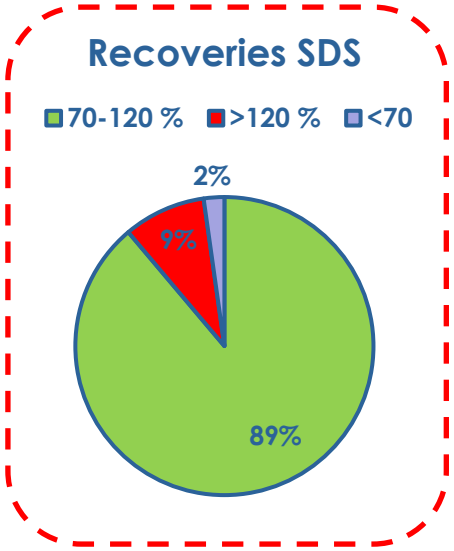
- Positive ion spray voltage: 3500 V
- Negative ion spray voltage: 2500 V
- Sheath gas: 50
- Aux gas: 10
- Sweep gas: 1
- Ion transfer tube temperature: 25 °C
- Vaporiser temperature: 350 °C

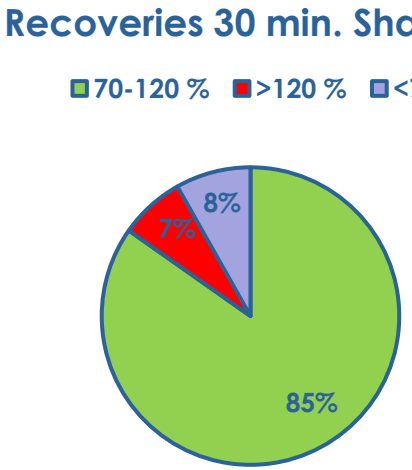
Pesticide residues evaluated

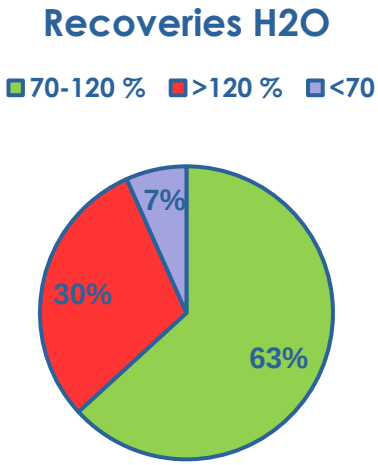


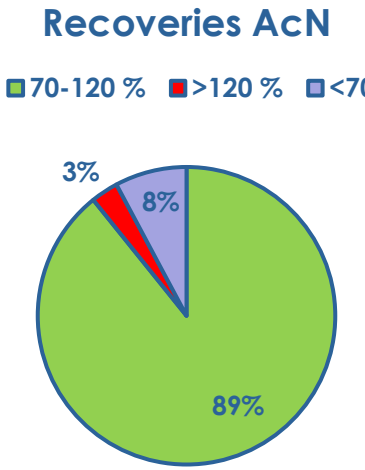
- The AcN method detected the fewest compounds (258).
- There is no difference between the hydration test with 6 minutes of shaking and with 30 minutes (260).
- The method with SDS detects the most compounds (268)

Pesticide residues evaluated



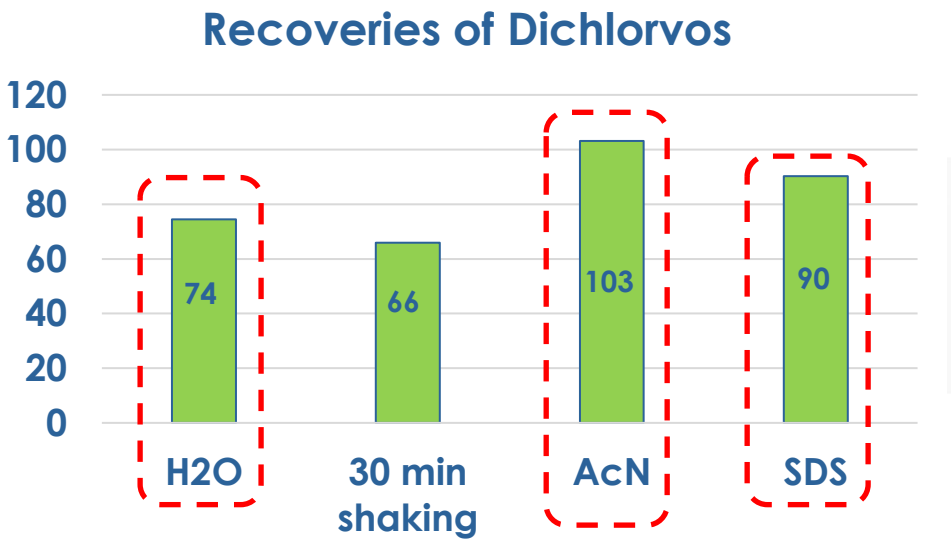




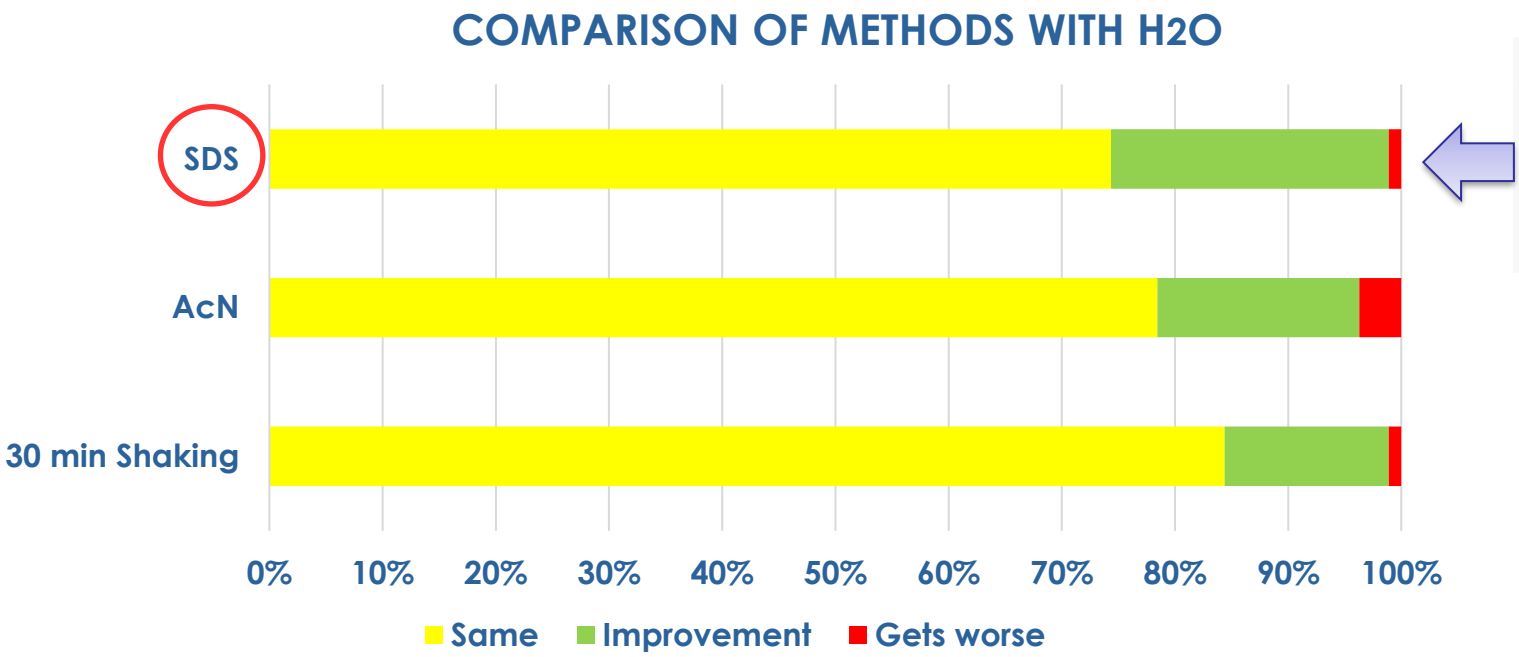


- The AcN method detected the fewest compounds (258) In recoveries it is similar to SDS
- Hydrating with 30 minutes of agitation the recoveries are better than with 6 minutes.
- SDS has good recoveries and is the method that has detected the most compounds.

Pesticide residues evaluated



Pesticide residues evaluated



Conclusion

- SDS is an extraction method that improves on the method of hydrating the sample with H2O
- Longer agitation time has an impact on improving recoveries
- Without hydrating the sample, the number of detected compounds is lower, however recovery of Dichlorvos is better

Thank You
for Your Attention