Automated pressurised sample extraction as an effective tool in the analysis of difficult matrices

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European Commission

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Sample hydration: pros and cons

- Document No. SANTE/12682/2019 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
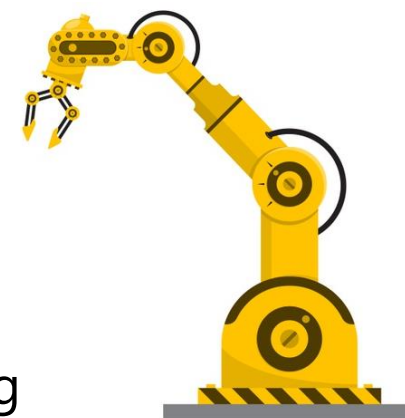
Sample hydration: pros and cons

- Water must be removed in a later step, increasing consumable expenses and time
  - *e.g. magnesium sulphate, sodium sulphate, calcium chloride.*

- Energetic extraction conditions must be employed if no sample hydration is to be employed

- These are generally outside the capabilities of standard extraction techniques in laboratories

Solution?

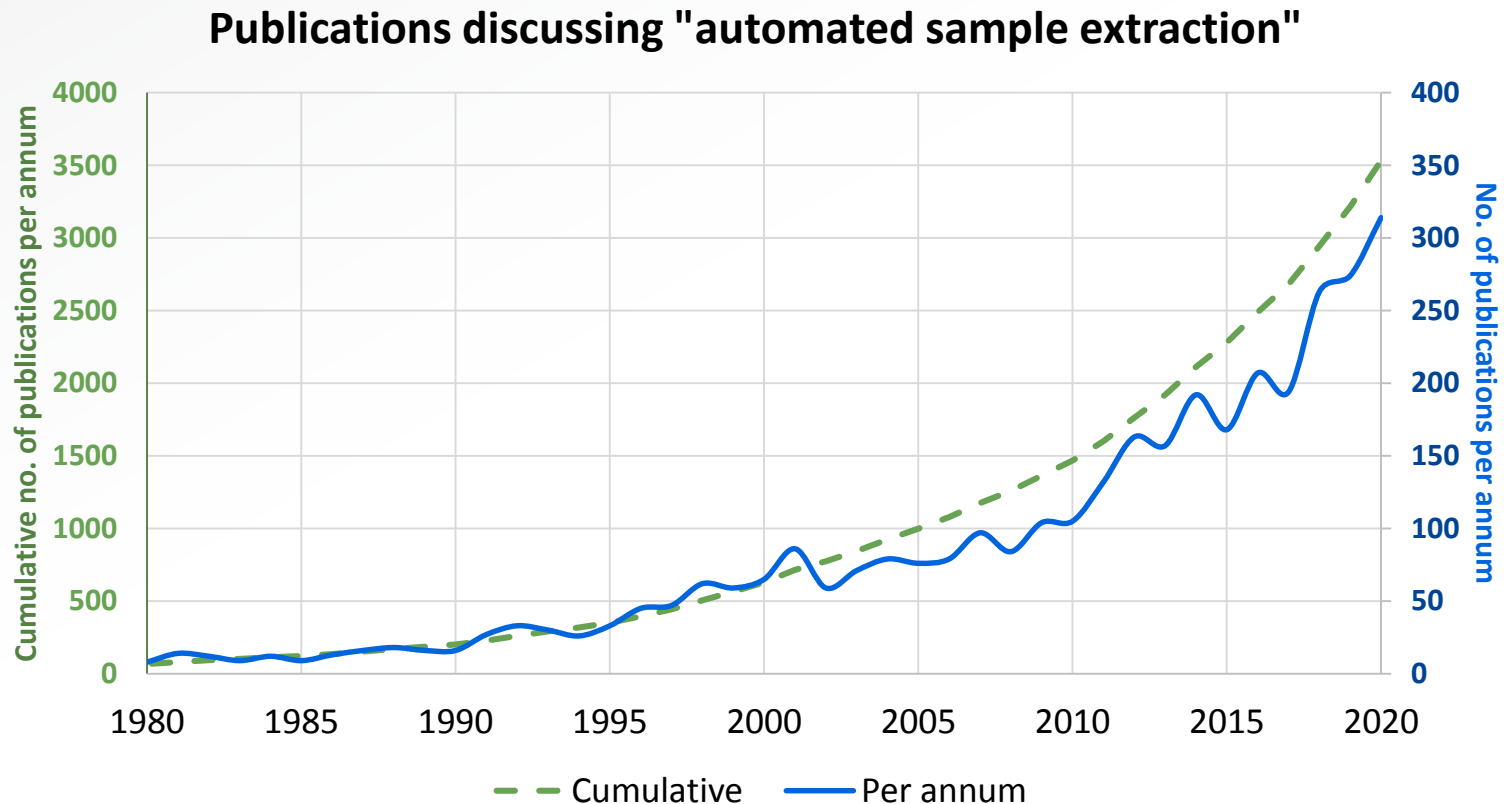**High energetic extraction with organic solvents**

E. g. Automated pressurised liquid extraction and heating
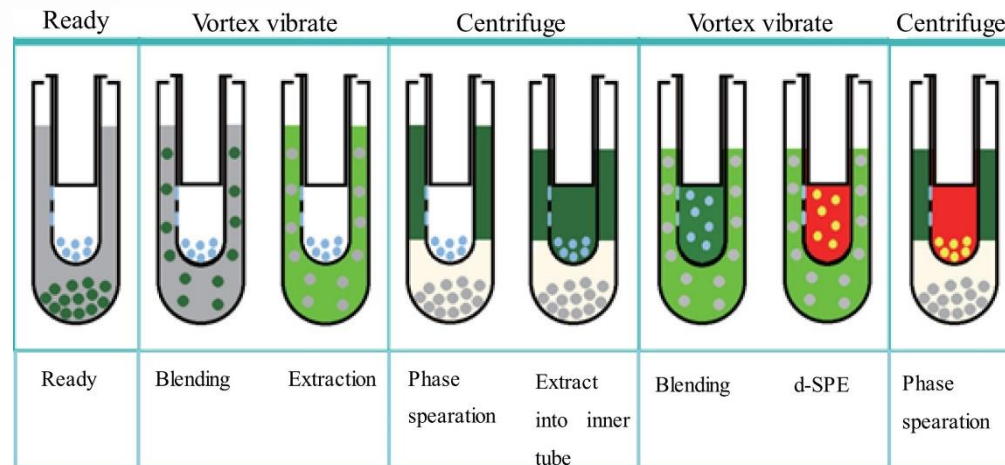
Sample extraction automation

- Automated extraction is attracting interest from laboratories
  - Increased robustness, reproducibility and potential time and cost reduction

Publications discussing "automated sample extraction"
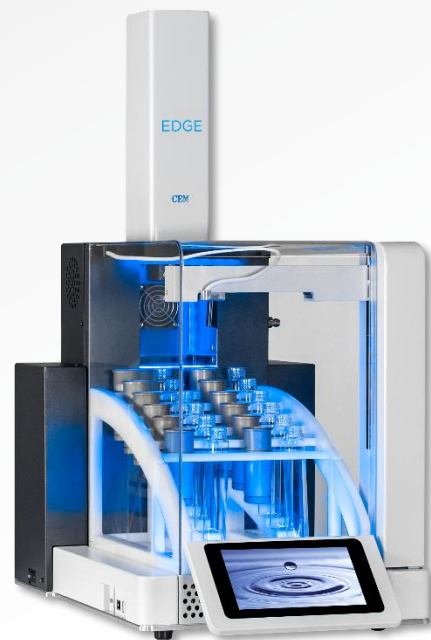
Sample extraction automation

- Automated extraction is attracting interest from laboratories
  - Increased robustness, reproducibility and potential time and personnel cost reduction

- Automatic shakers have been increasingly gaining popularity (e. g. Agytax, GenoGrindr)

- Attempts at automating popular manual extraction methods, e. g. QuEChERS

Commercially available instrumentation

ANKOM FLEX
Analyte Extractor

CEM EDGE®
Automated Solvent
Extraction System

FMS PLE®
And SuperVap®
Concentrator

Dionex ASE®
Accelerated
Solvent Extraction

Commercially available instrumentation

ANKOM FLEX
Analyte Extractor

CEM EDGE®
Automated Solvent
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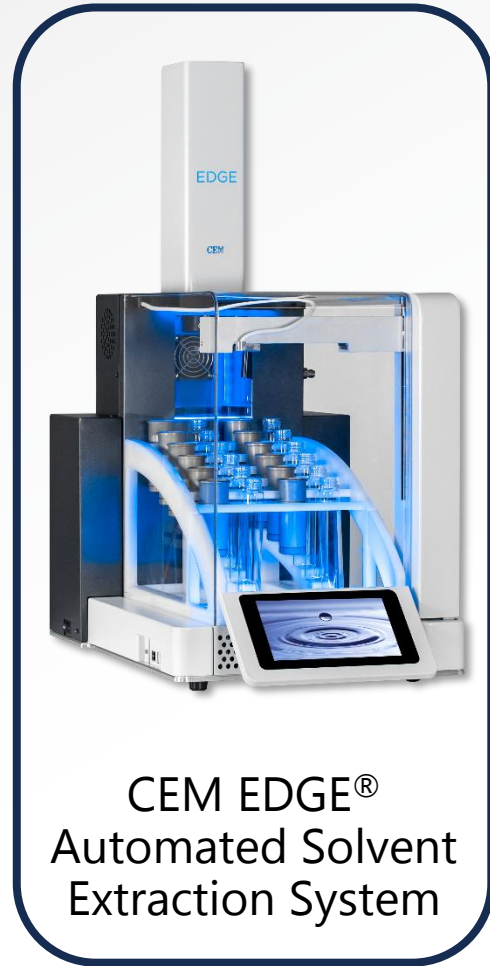
FMS PLE®
And SuperVap®
Concentrator

Dionex ASE®
Accelerated
Solvent Extraction

EURL-FV

Automated extraction: method optimization

Method (AMXX)	Solvent	Volume (mL)	Bubbling time (s)	Hold time (s)	T (° C)	Rinse step	Rinse volume (mL)	Total solvent (mL)	Dilution factor (V/m)	Clean-up (dSPE)
AM01	AcN	10	-	120	40	No	-	10	2.50	-
AM02	AcN	10	-	120	40	No	-	10	2.50	PSA
AM03	AcN	10	-	120	40	No	-	10	2.50	PSA, FA
AM04	AcOEt	10	-	120	40	No	-	10	2.50	-
AM05	AcOEt	10	-	120	40	No	-	10	2.50	PSA
AM06	AcOEt	10	-	120	40	No	-	10	2.50	PSA, FA
AM07	AcN	10	60	60	40	No	-	10	2.50	-
AM08	AcN	10	90	60	40	No	-	10	2.50	-
AM09	AcN	5	60	60	40	Yes	5	10	2.50	-
AM10	AcN	10	-	90	40	Yes	5	15	3.75	-
AM11	AcN	10	30	90	40	Yes	5	15	3.75	-
AM12	AcN	10	-	150	40	Yes	5	15	3.75	-

Sample is rinsed with additional solvent, and afterwards, the instrument is automatically cleaned and will begin the extraction of the next sample in the batch
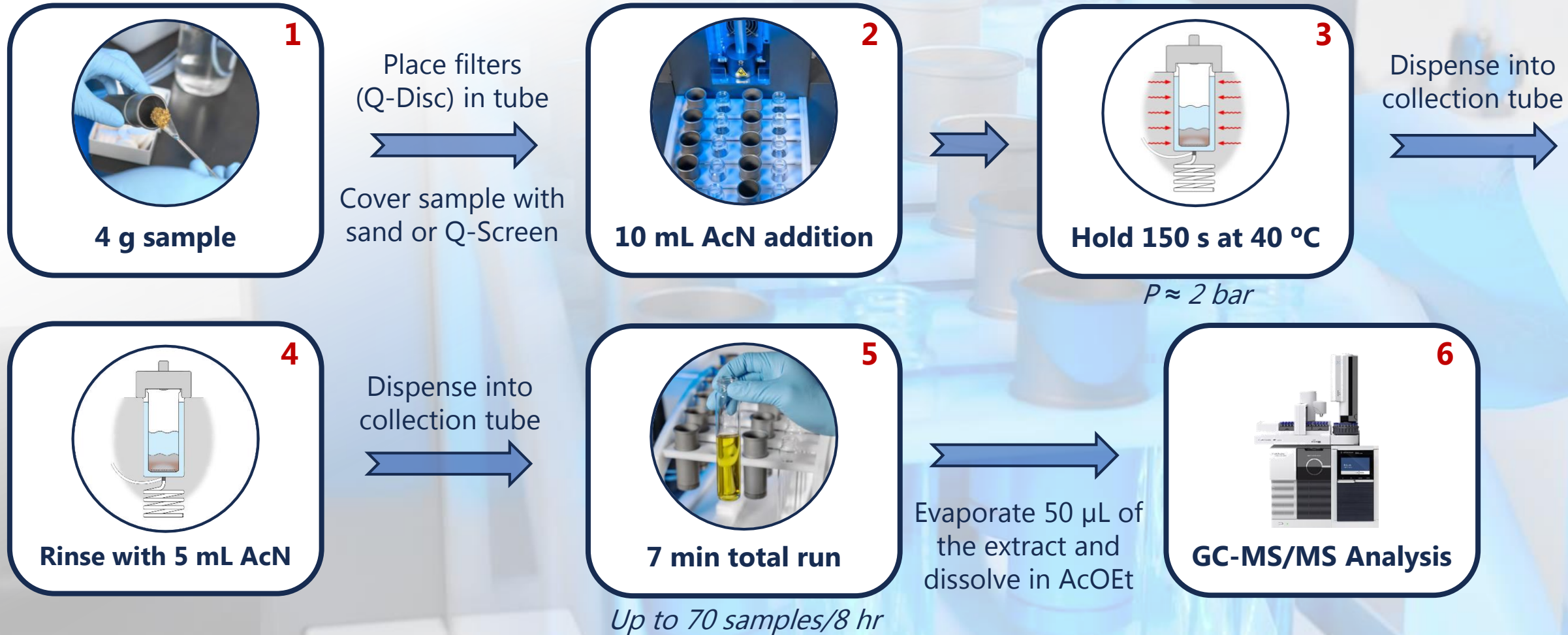
2x video playback

- AcN was the most efficient solvent

- Bubbling (agytation) was deemed counterproductive

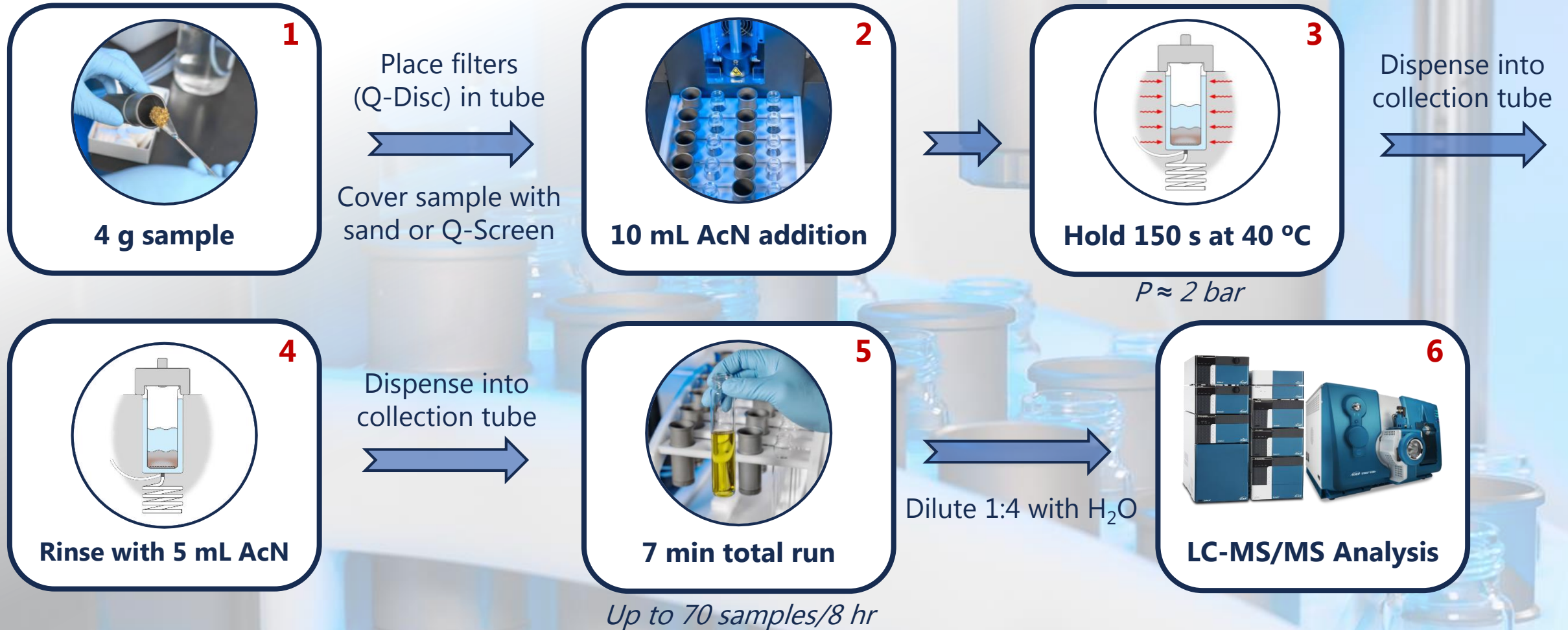- A rinse step significantly improved recovery values

Cocoa and coffee: extraction & GC analysis

1 4 g sample

Place filters (Q-Disc) in tube

Cover sample with sand or Q-Screen

2 10 mL AcN addition

3 Hold 150 s at 40 ºC

P ≈ 2 bar

Dispense into collection tube

4 Rinse with 5 mL AcN

Dispense into collection tube

5 7 min total run

Up to 70 samples/8 hr

Evaporate 50 µL of the extract and dissolve in AcOEt
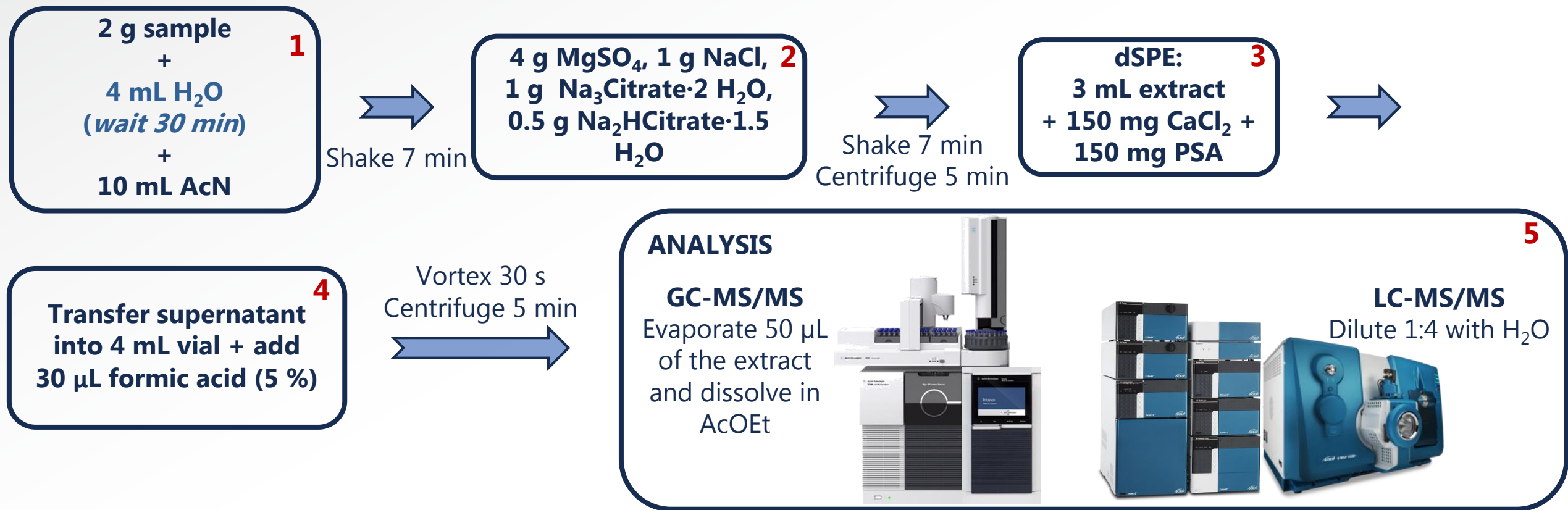
6 GC-MS/MS Analysis

Díaz-Galiano, F. J.; Murcia-Morales, M.; Gómez-Ramos, M. M.; Ferrer, C.; Fernández-Alba, A.R. Presence of anthraquinone in coffee and tea samples. An improved methodology based on mass spectrometry and a pilot monitoring programme. *Anal. Methods* **2021**, *13,* 99-109.

Cocoa and coffee: extraction & LC analysis

1
4 g sample

Place filters (Q-Disc) in tube

Cover sample with sand or Q-Screen

2
10 mL AcN addition

3
Hold 150 s at 40 °C

P ≈ 2 bar

Dispense into collection tube

4
Rinse with 5 mL AcN

Dispense into collection tube

5
7 min total run

Up to 70 samples/8 hr

Dilute 1:4 with H_2O

6
LC-MS/MS Analysis

Díaz-Galiano, F. J.; Murcia-Morales, M.; Gómez-Ramos, M. M.; Ferrer, C.; Fernández-Alba, A.R. Presence of anthraquinone in coffee and tea samples. An improved methodology based on mass spectrometry and a pilot monitoring programme. *Anal. Methods* **2021**, *13,* 99-109.

Manual extraction: coffee, cocoa, and tea

- Sample hydration causes the coextraction of matrix components that hinder the analysis
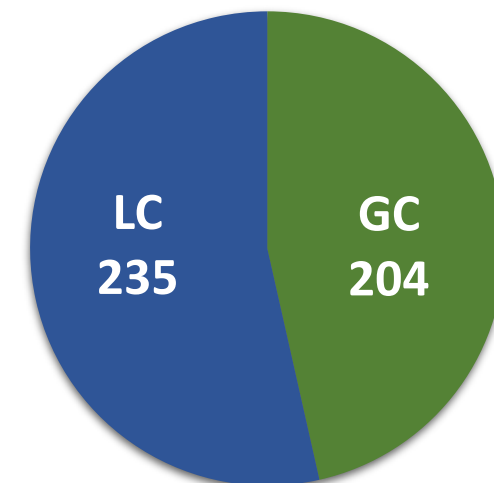
1
2 g sample
+
4 mL H_2O
(*wait 30 min*)
+
10 mL AcN

Shake 7 min

2
4 g $MgSO_4$, 1 g NaCl,
1 g Na_3Citrate·2 H_2O,
0.5 g Na_2HCitrate·1.5 H_2O

Shake 7 min
Centrifuge 5 min

3
dSPE:
3 mL extract
+ 150 mg $CaCl_2$ +
150 mg PSA

4
Transfer supernatant
into 4 mL vial + add
30 μL formic acid (5 %)

Vortex 30 s
Centrifuge 5 min

5
ANALYSIS

GC-MS/MS
Evaporate 50 μL
of the extract
and dissolve in
AcOEt

LC-MS/MS
Dilute 1:4 with H_2O

Lozano, A.; Rajski, Ł.; Belmonte-Valles, N.; Uclés, A.; Uclés, S.; Mezcua, M.; Fernández-Alba, A.R. Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *J. Chromatogr. A* **2012**, *1268,* 109–122.

Cocoa and coffee: pesticide residues evaluated

- **363** unique pesticide residues were evaluated by LC and GC

- In sum, **235** pesticide residues were evaluated by **LC-QqQ-MS/MS** and **204** by **GC-QqQ-MS/MS**

- For pesticides both LC and GC amenable, validation was performed with both techniques

- Evaluation performed at 0.010 and 0.050 mg/kg
  - Mean recovery (n = 5)
  - Within-laboratory reproducibility expressed as RSD_r
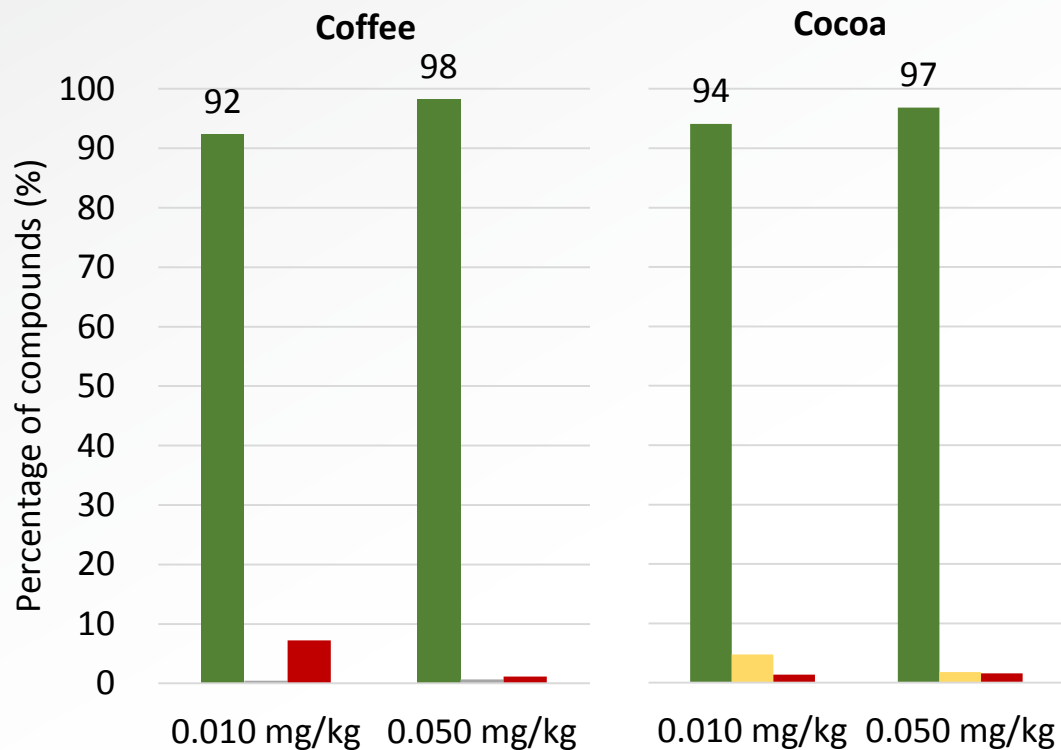 - Matrix effect was also studied

LC 235 / GC 204

Comparison between extraction methods

Automated extraction
(*Pressurized liquid extraction*)

Manual extraction
(*QuEChERS with hydration*)

Over 90 % of compounds successfully validated at 0.01 mg/kg with RSD_r ≤ 20 %

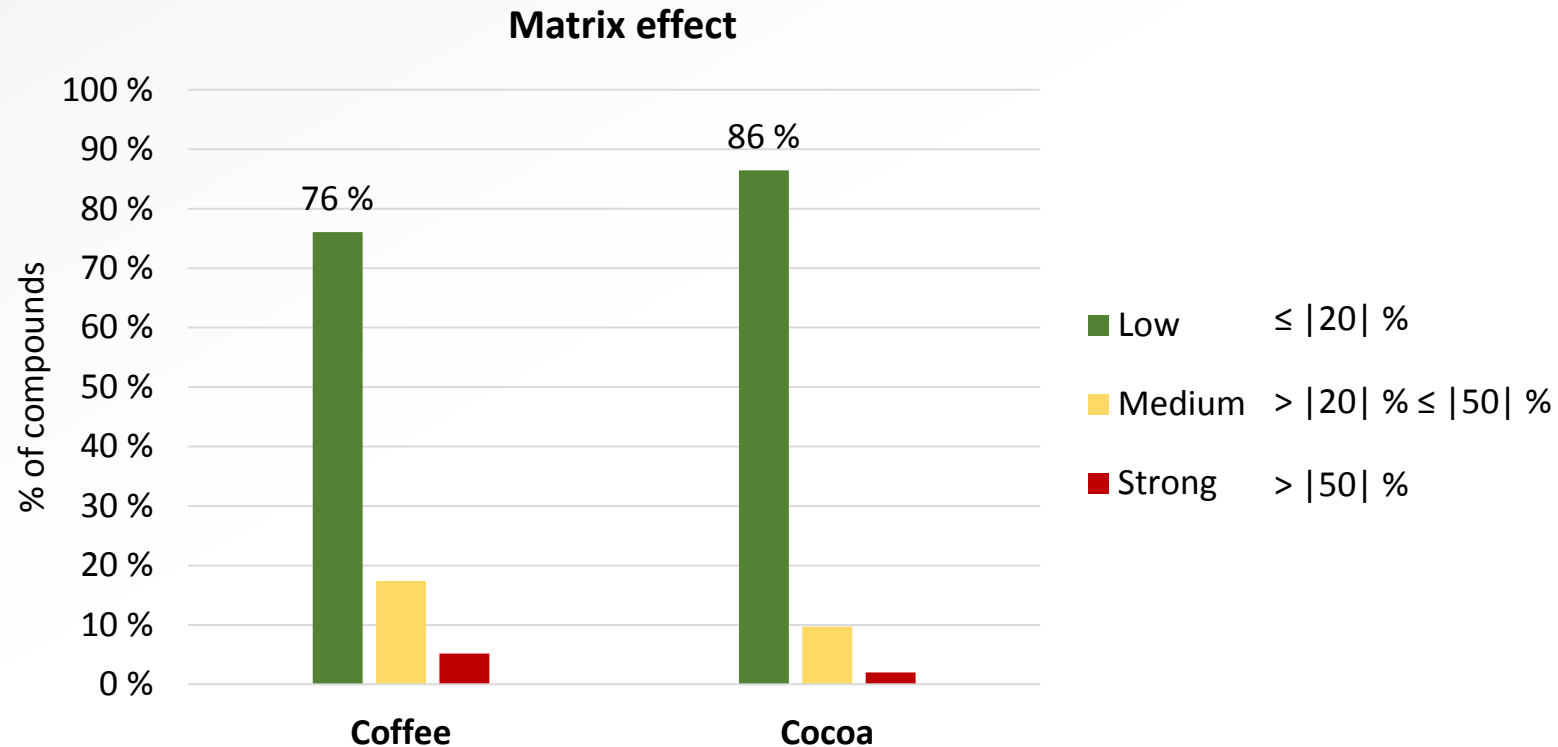Far fewer compounds could be successfully validated with this method, with a high number of non-detections

(a)   Not detected

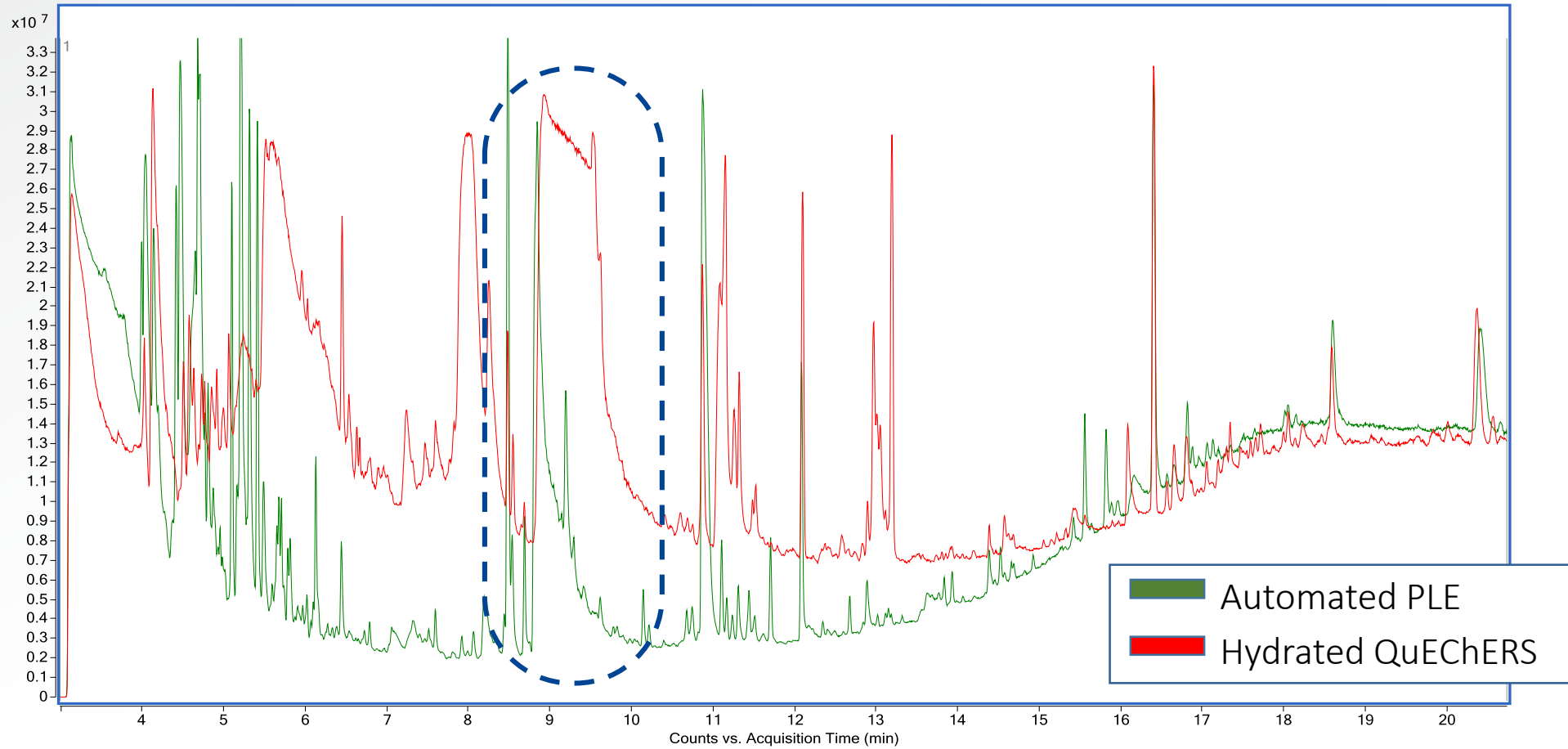(b)   Not fulfilling validation requirements

Automated method matrix effects

- Linearity and matrix effect were evaluated in the 0.005 – 0.200 mg/L range
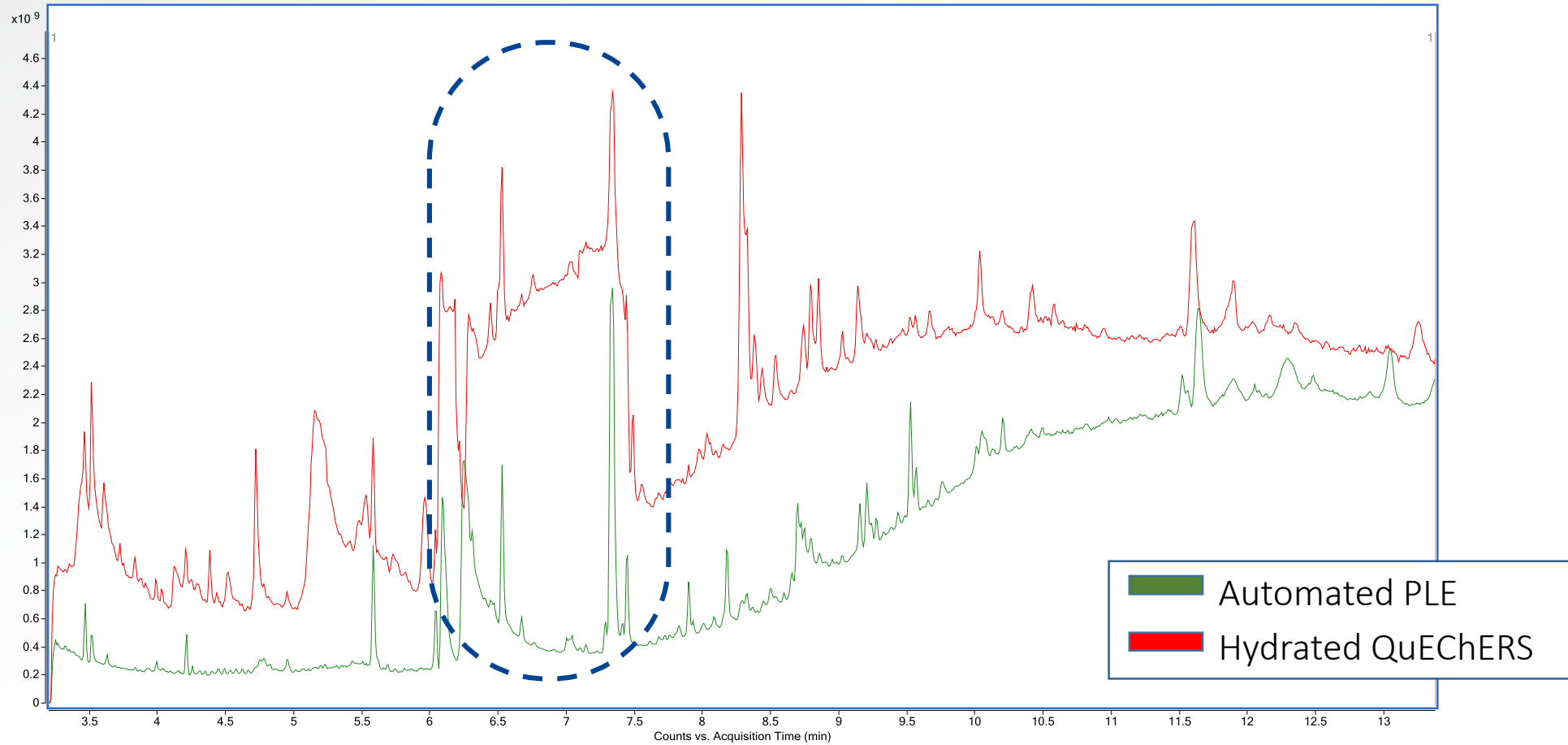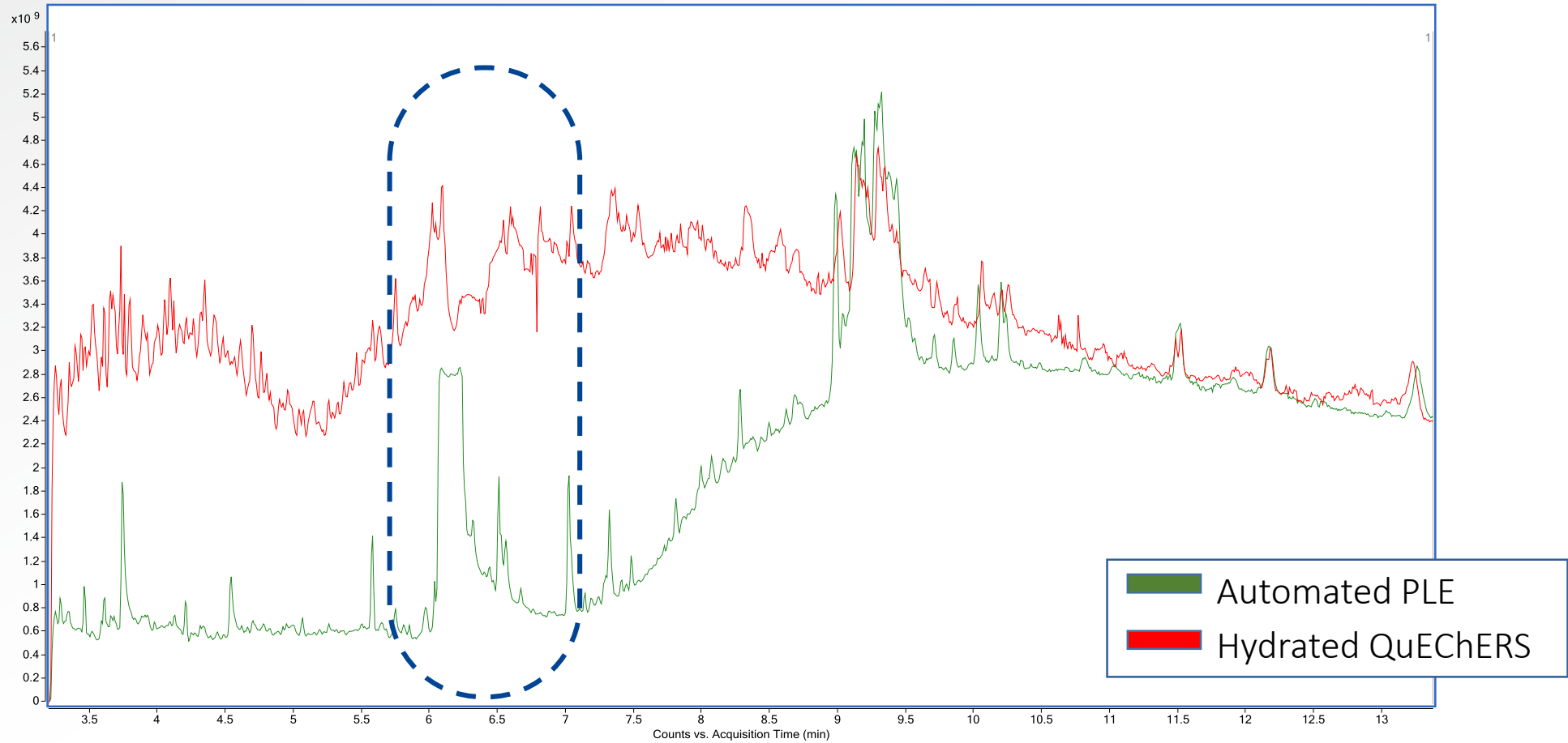 - Correlation coefficient was ≥ 0.99 in all successfully validated compounds

Matrix effect

Low   ≤ |20| %

Medium   > |20| % ≤ |50| %

Strong   > |50| %

Total ion chromatogram of tea

Total ion chromatogram of cocoa

Total ion chromatogram of coffee

Legend:
- Automated PLE
- Hydrated QuEChERS

Counts vs. Acquisition Time (min)
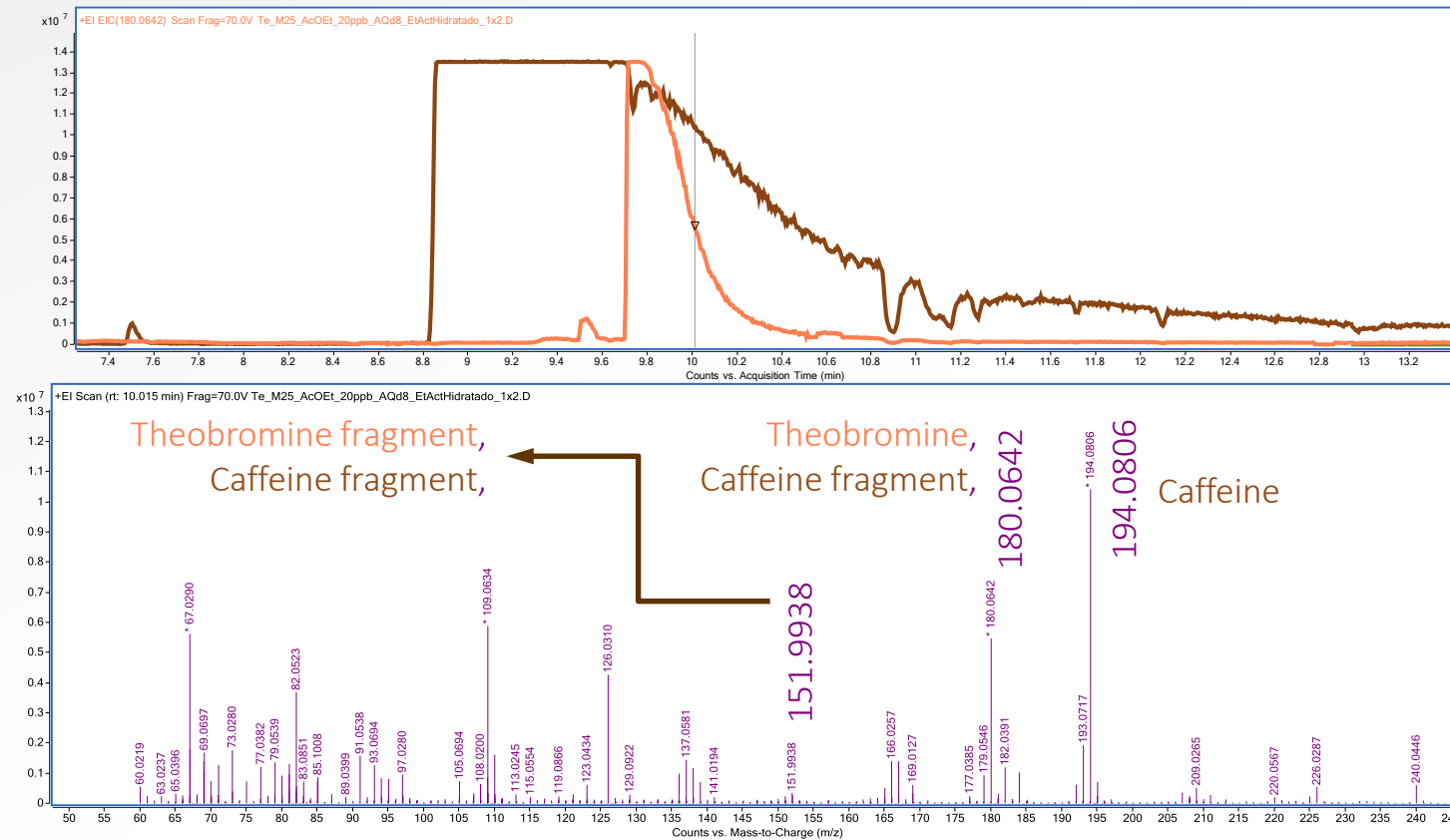
Main intereferences in hydrated methods

- Caffeine and theobromine have been identified as the main coextracted matrix interferences using an Agilent 7250 GC/Q-TOF HRAMS instrument
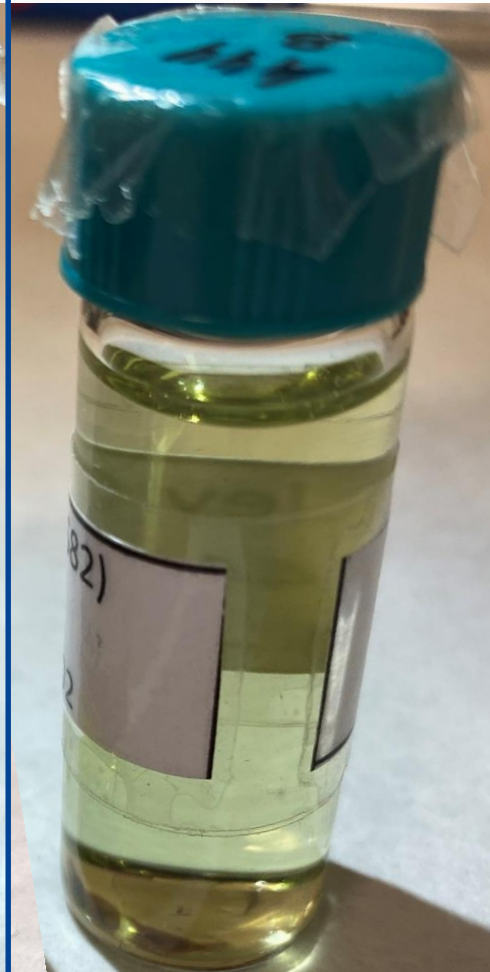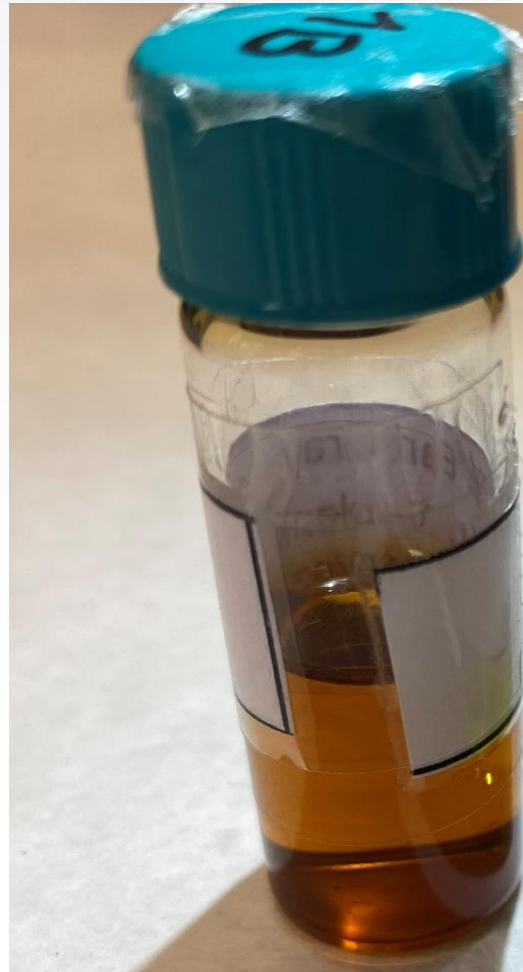
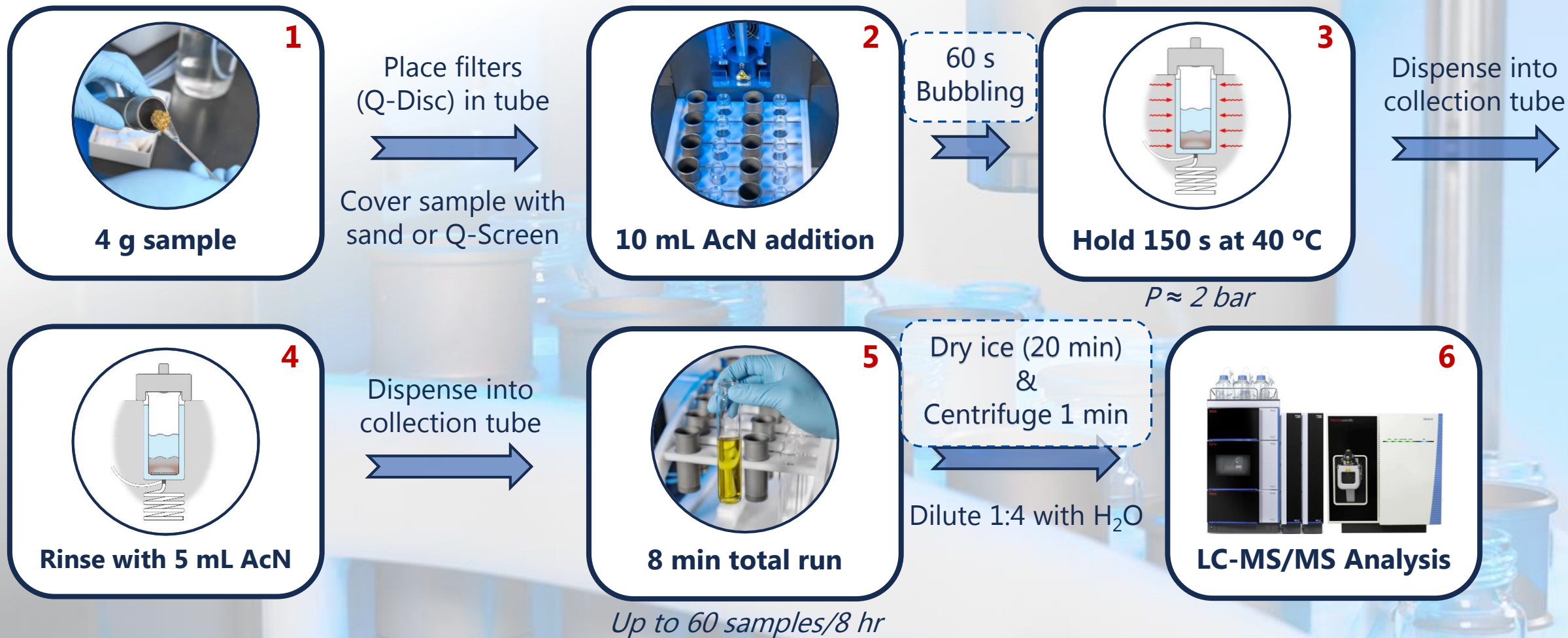# Final acetonitrile extracts visual comparison in tea

Manual extraction

Automated PLE extraction
(EDGE)

Raw olives: extraction & Dual Channel LC analysis

1 — 4 g sample

Place filters (Q-Disc) in tube

Cover sample with sand or Q-Screen

2 — 10 mL AcN addition

60 s Bubbling

3 — Hold 150 s at 40 ºC

$P \approx 2$ bar

Dispense into collection tube

4 — Rinse with 5 mL AcN

Dispense into collection tube

5 — 8 min total run

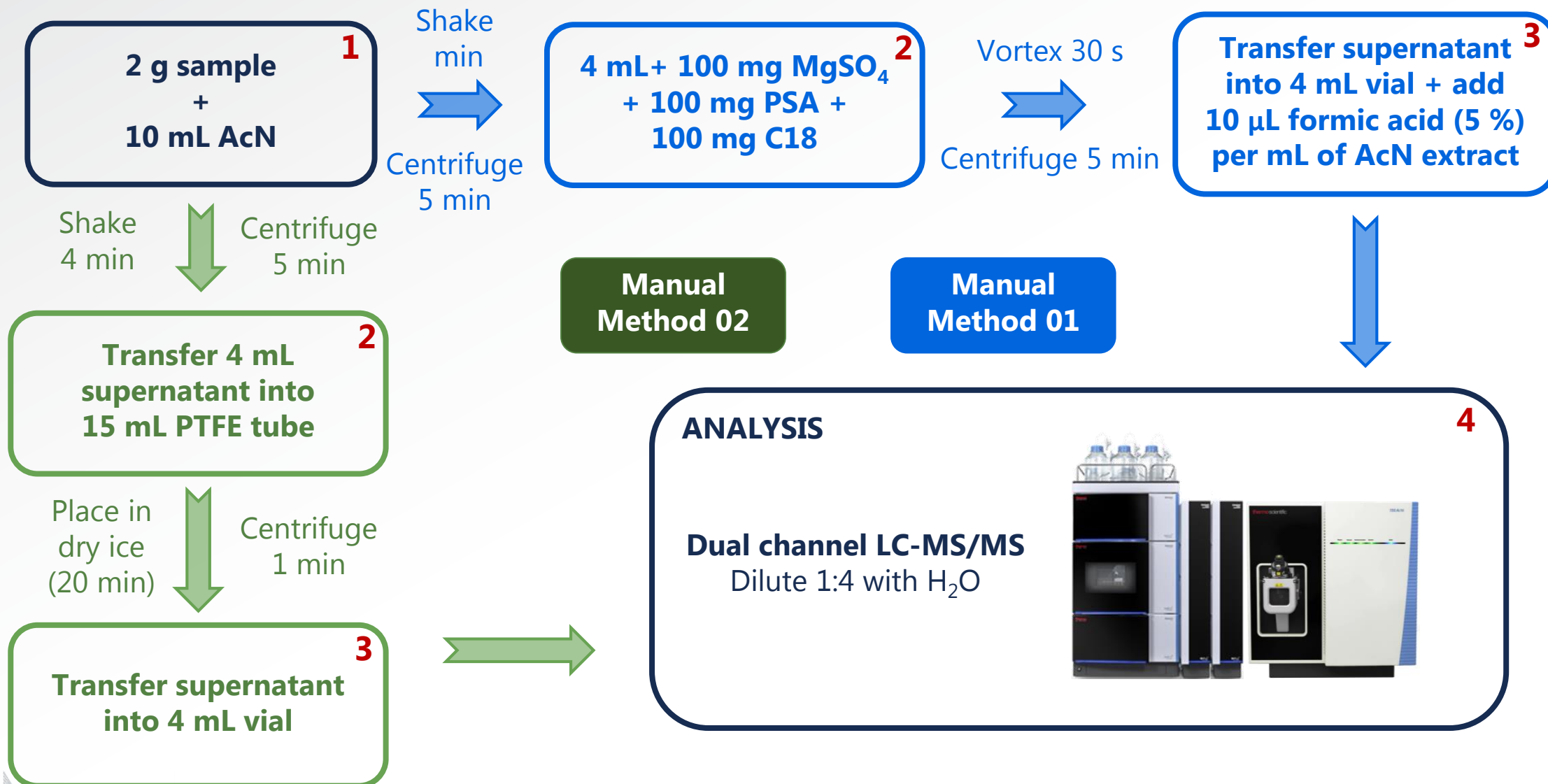Up to 60 samples/8 hr

Dry ice (20 min) & Centrifuge 1 min

Dilute 1:4 with H_2O

6 — LC-MS/MS Analysis

Manzano-Sánchez, L.; Rajski, Ł.; Díaz-Galiano, F.J.; Fernández-Alba, A. R. EURL-FV (2020-M39) Development and validation of a multiresidue method for high oil and intermediate water content commodities: olives.
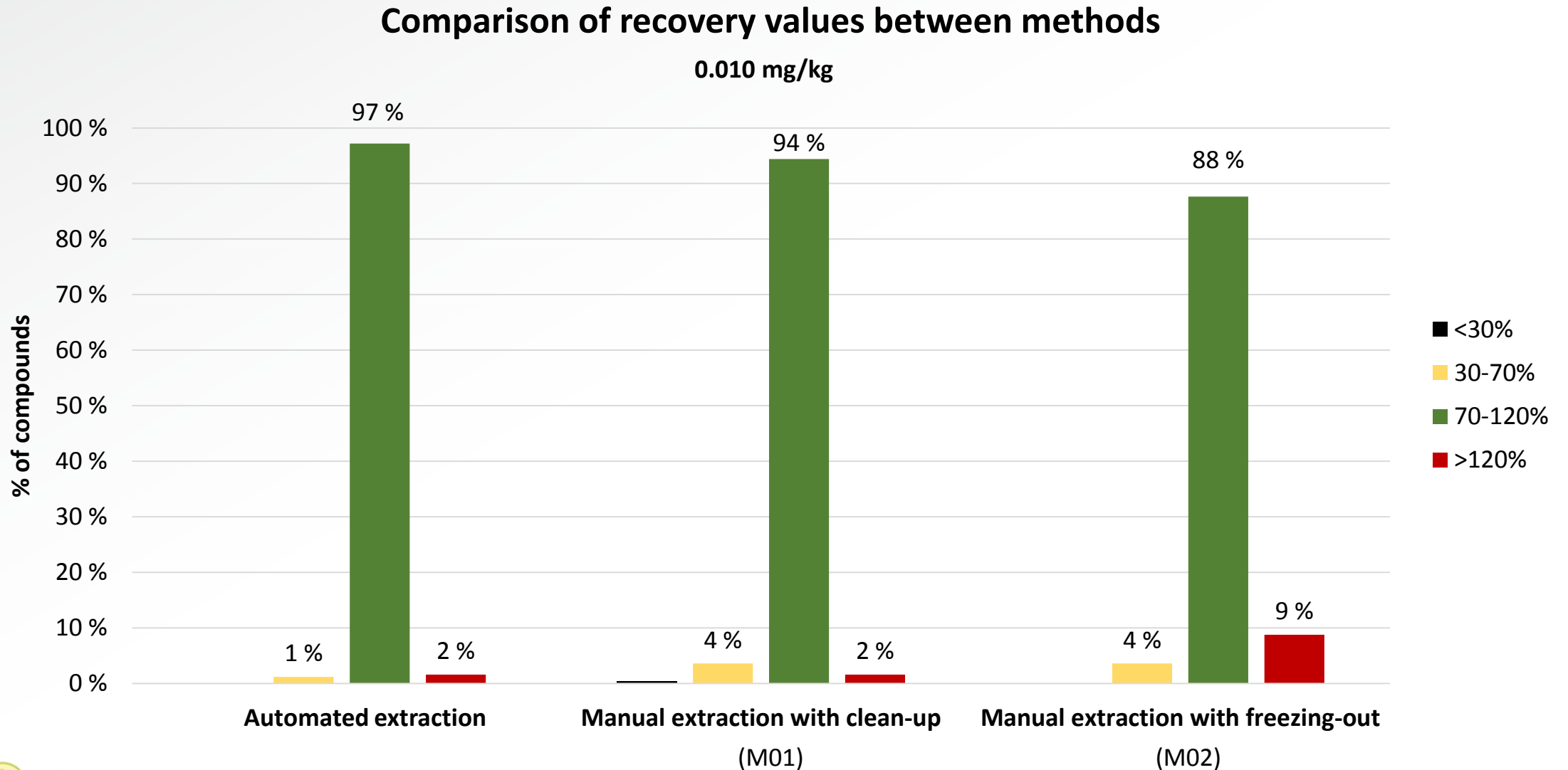
Manual extraction methods: raw olives

2 g sample + 10 mL AcN   **1**

Shake min → Centrifuge 5 min

4 mL+ 100 mg MgSO$_4$ + 100 mg PSA + 100 mg C18   **2**

Vortex 30 s → Centrifuge 5 min

Transfer supernatant into 4 mL vial + add 10 µL formic acid (5 %) per mL of AcN extract   **3**

Shake 4 min / Centrifuge 5 min

Manual Method 02

Manual Method 01

Transfer 4 mL supernatant into 15 mL PTFE tube   **2**

Place in dry ice (20 min) / Centrifuge 1 min

Transfer supernatant into 4 mL vial   **3**

ANALYSIS   **4**

Dual channel LC-MS/MS
Dilute 1:4 with H$_2$O

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Raw olives: automated and manual extraction

Comparison of recovery values between methods

0.010 mg/kg

Legend:
- ■ <30%
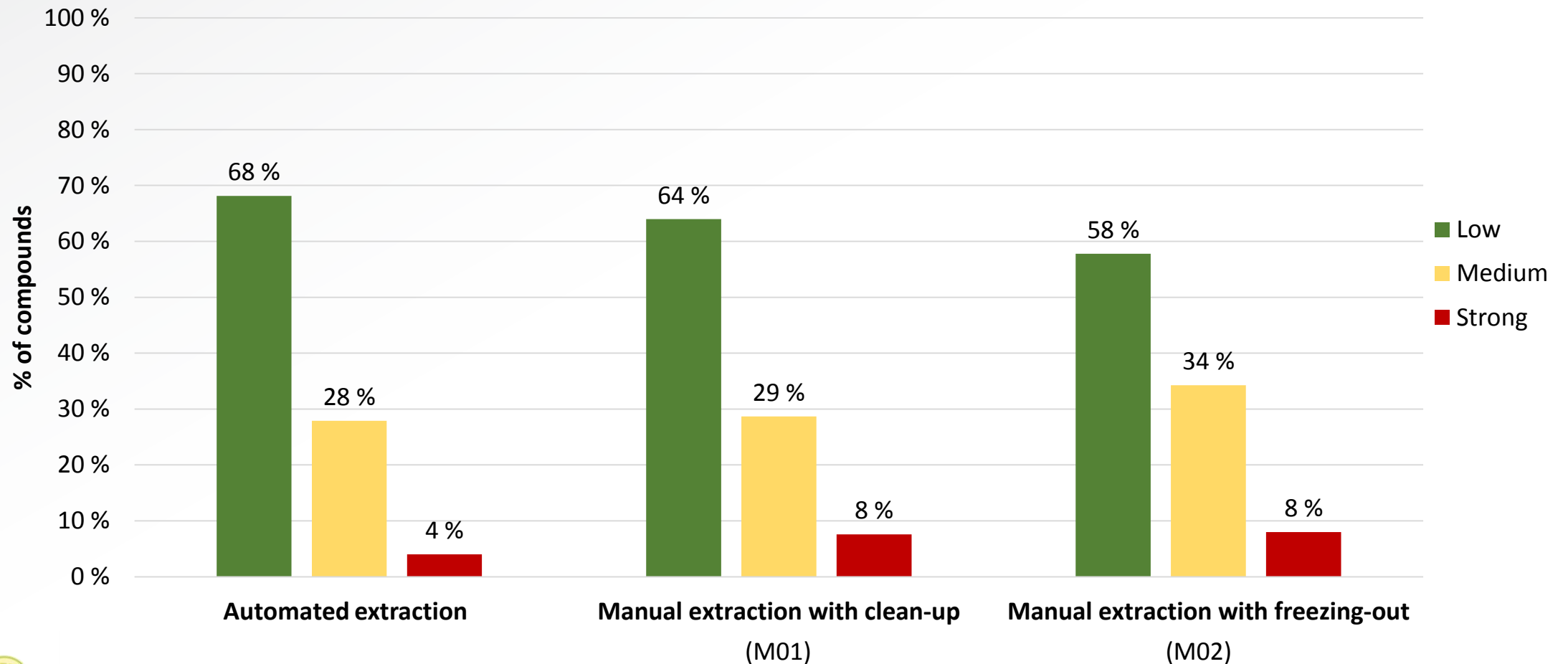- ■ 30-70%
- ■ 70-120%
- ■ >120%

Raw olives: automated and manual extraction

Comparison of matrix effects between methods

0.010 mg/kg

Comparison of olive final extracts

Automated PLE extraction

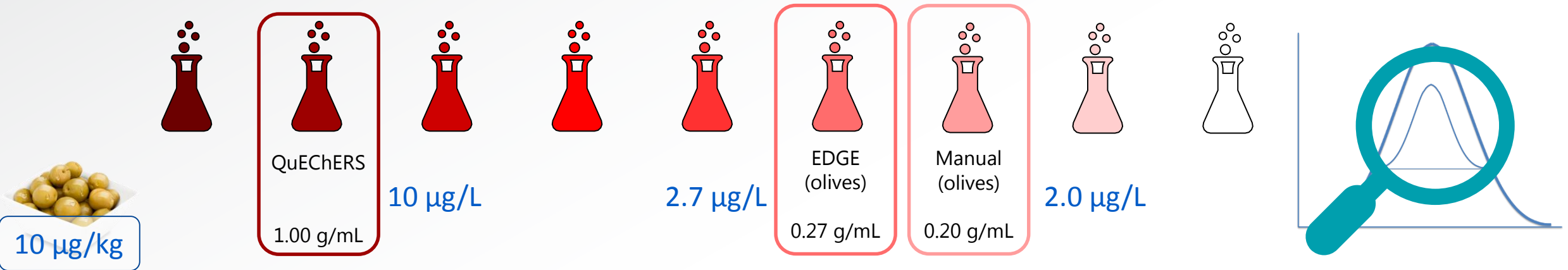(EDGE, freezing-out)

Manual extraction 01

(PSA + C18 + MgSO$_4$)

Manual extraction 02

(freezing-out)

- The proposed methods significantly dilute extracted samples

10 µg/kg

QuEChERS
1.00 g/mL

10 µg/L

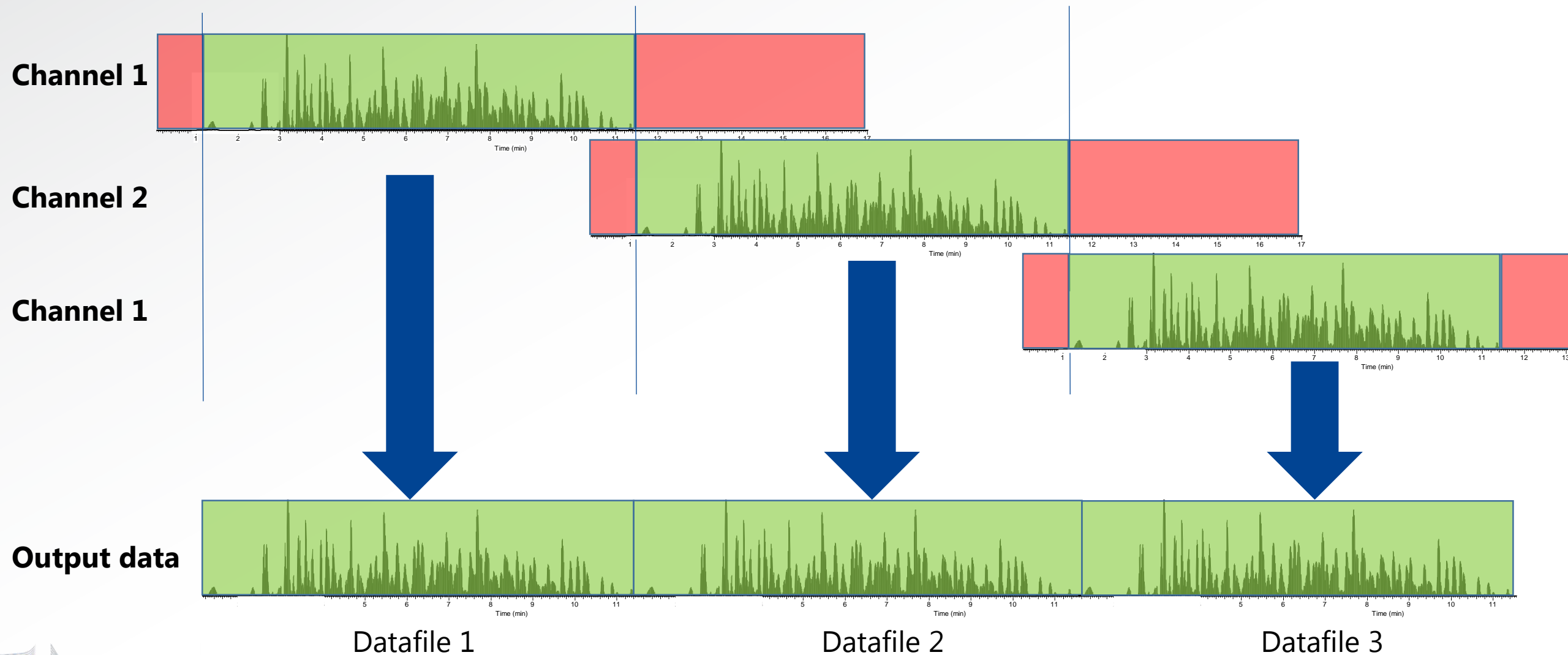2.7 µg/L

EDGE (olives)
0.27 g/mL

Manual (olives)
0.20 g/mL

2.0 µg/L

- To overcome dilution difficulties, sensitive analytical instrumentation is required

Sample throughput: dual channel LC-MS/MS

Datafile 1

Datafile 2

Datafile 3

Sample throughput: dual channel LC-MS/MS

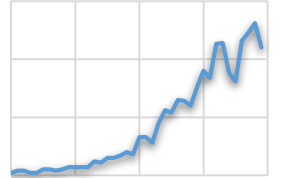**Number of injections per unit of time**

- With dual channel chromatography, pre-acquisition and post-acquisition MS-idle times are removed

- Sample throughput is increased over 70 % (45 injections in an 8 hr period)

- Automated extraction procedures provide comparable sample throughput (up to 70 samples / 8 hr)

Conclusions

- Interest in automation within laboratories has increased in recent years

- Pressurized liquid extraction is a viable alternative for sample extraction of matrixes traditionally subjected to a hydration step

- Automated pressurized liquid extraction overcomes the issues associated with QuEChERS extraction of pesticide residues from coffee beans, cocoa beans, tea and other dry herbs, and olives

Conclusions: advantages of PLE (EDGE)

- Sample throughput is as high as 70 samples per 8 h with the developed method

- Replaces tedious, manual extraction procedures

- No need for a clean-up step: the EDGE extracts can be directly injected

- Possibility of "bubbling" with an inert gas

- Thorough traceability: _who_ ran the sample, _when_ was the sample run, _what_ were the extraction conditions, and the possibility to _export_ all the data to a computer

References

- EURL-FV (2020-M39) Development and validation of a multiresidue method for high oil and intermediate wáter content commodities: olives.

- EURL-FV (2019-M34) Development and validation of a Multiresidue Method for high fat content commodities: coffee and cocoa beans.

- Díaz-Galiano, F. J.; Murcia-Morales, M.; Gómez-Ramos, M. M.; Ferrer, C.; Fernández-Alba, A.R. Presence of anthraquinone in coffee and tea samples. An improved methodology based on mass spectrometry and a pilot monitoring programme. *Anal. Methods* **2021**, *13,* 99-109.

- Lozano, A.; Rajski, Ł.; Belmonte-Valles, N.; Uclés, A.; Uclés, S.; Mezcua, M.; Fernández-Alba, A.R. Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *J. Chromatogr. A* **2012**, *1268,* 109–122.

Thank you for your attention