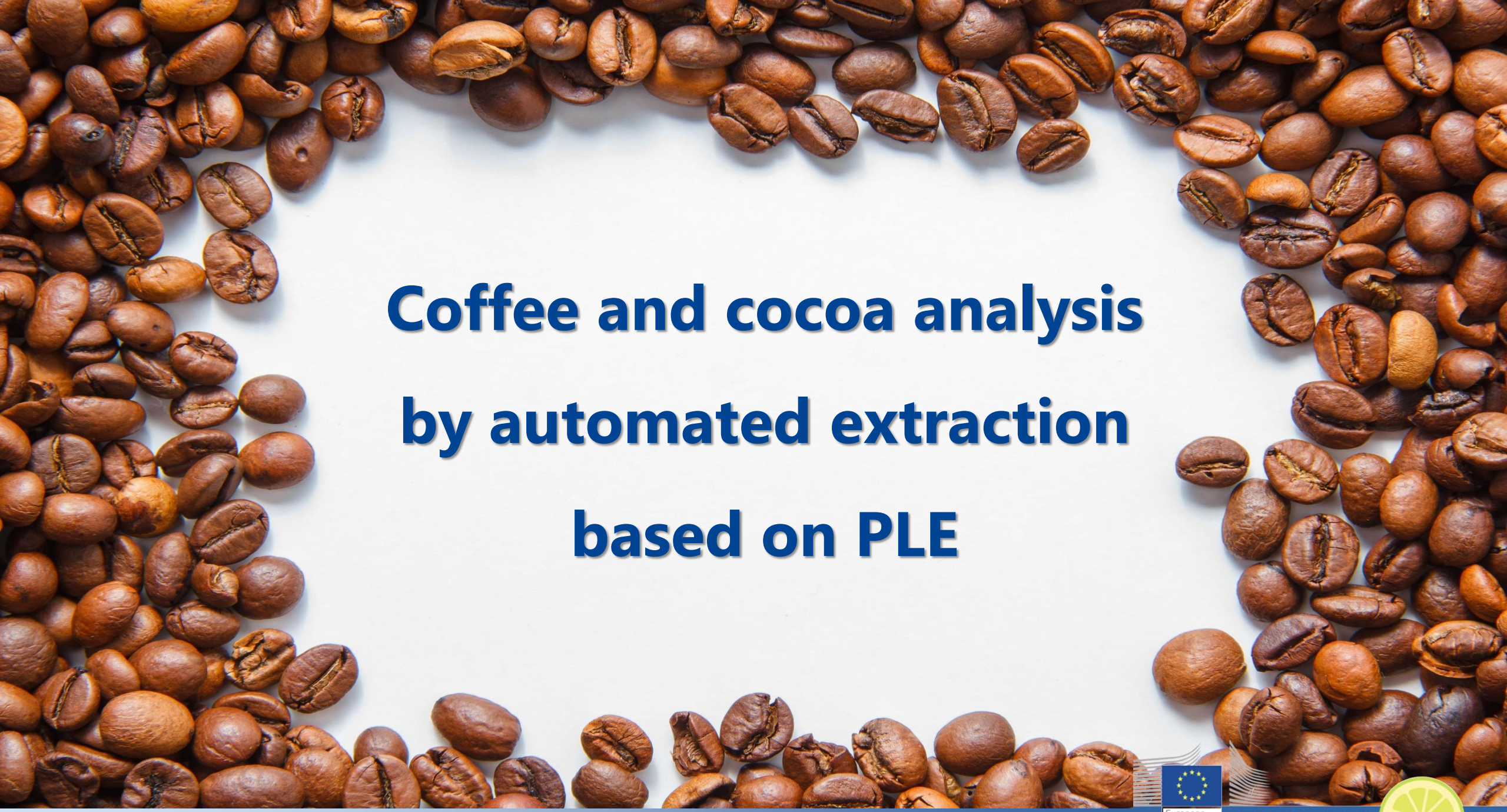


Coffee and cocoa analysis by automated extraction based on PLE

Sample hydration: pros and cons

- 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

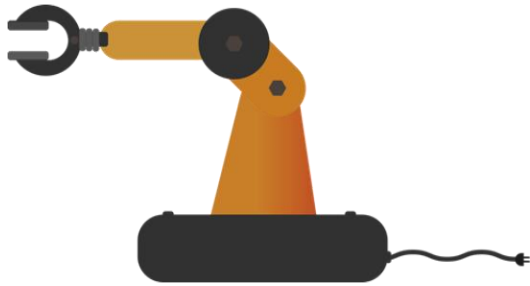
Sample hydration: pros and cons

- Water must be removed in a later step, increasing consumable expenses and time
- 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?

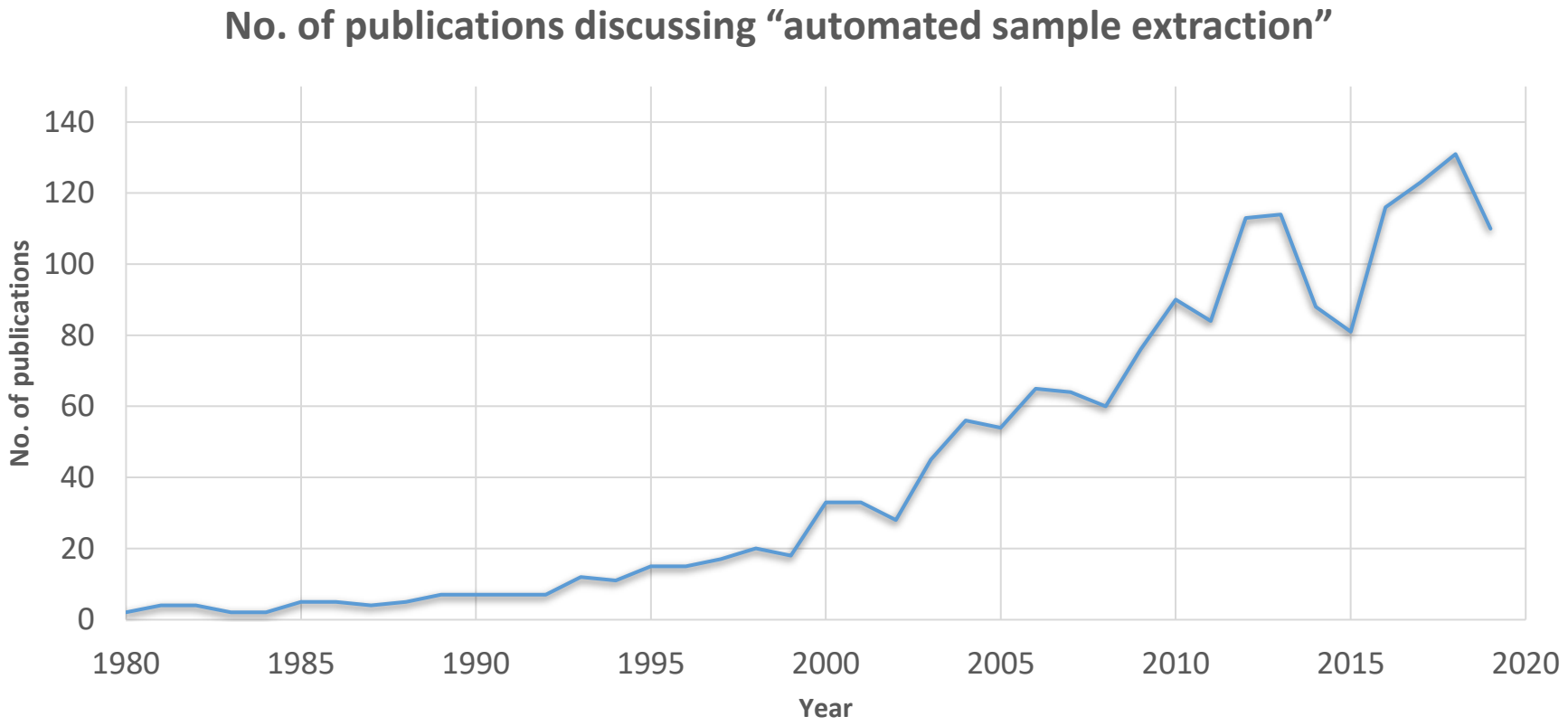
High energetic extraction with organic solvents

E. g. Automated pressurized liquid extraction and heating



Sample extraction automation

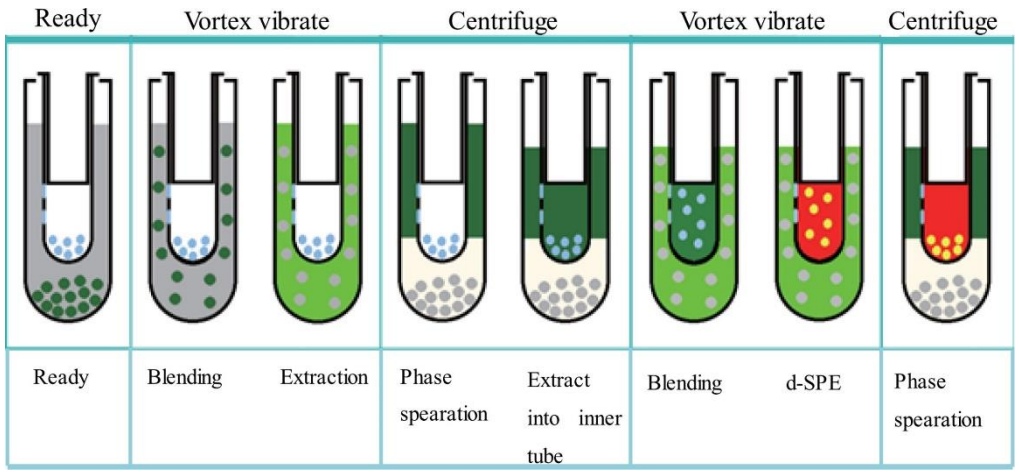
- Automated extraction is attracting interest from laboratories
  - Increased robustness, reproducibility and potential time and cost reduction



Source: Scopus search of “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

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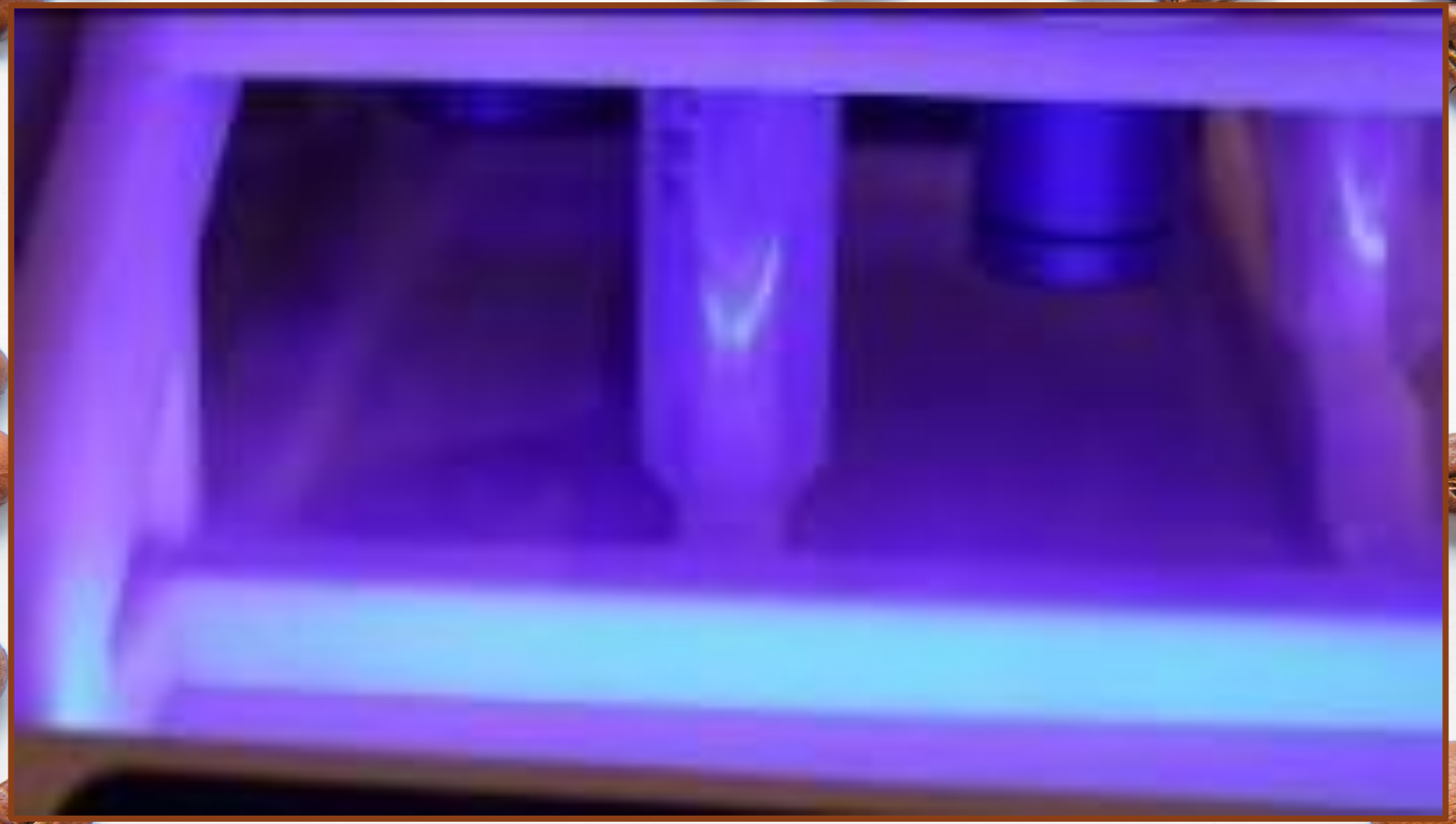
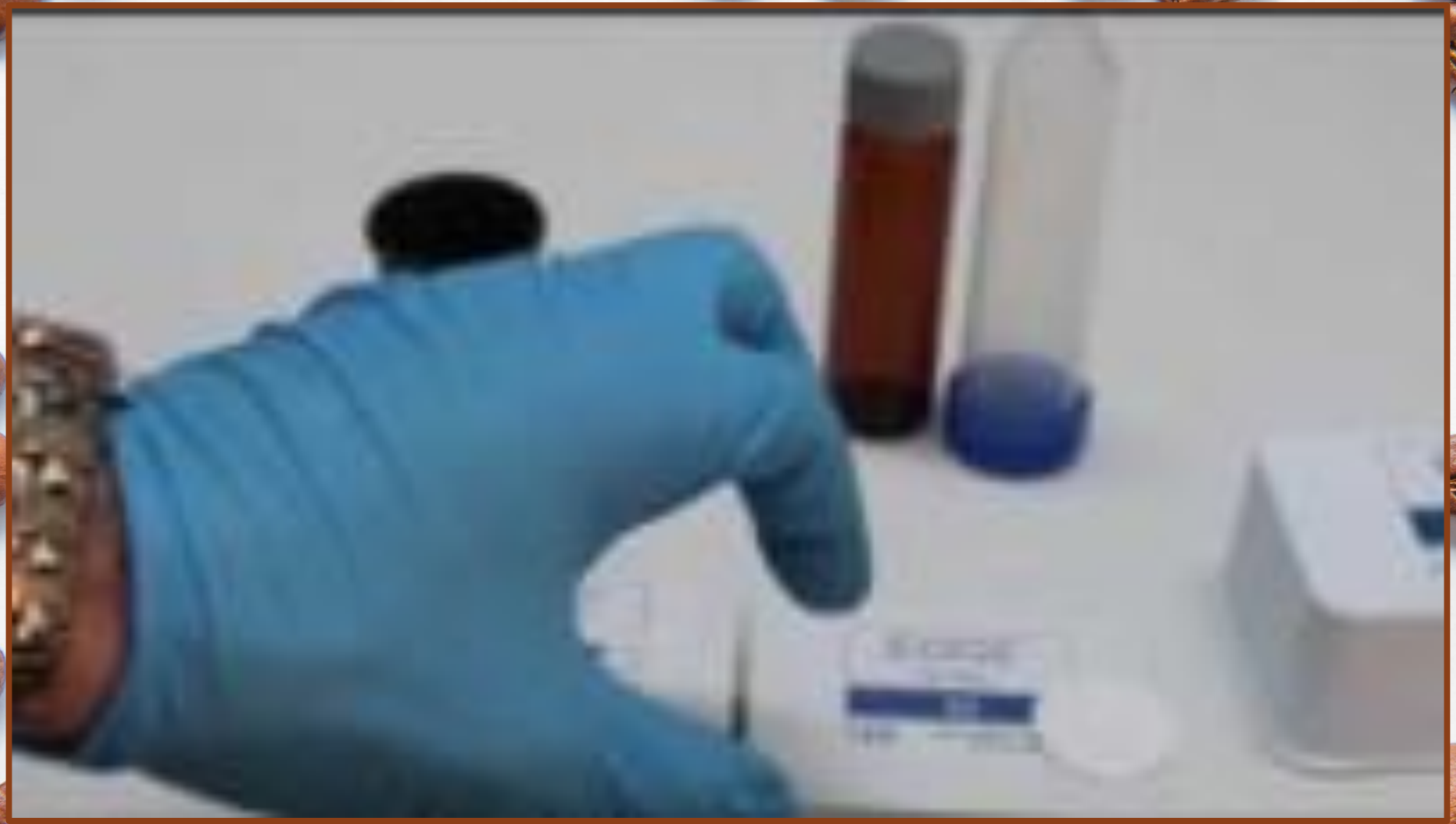
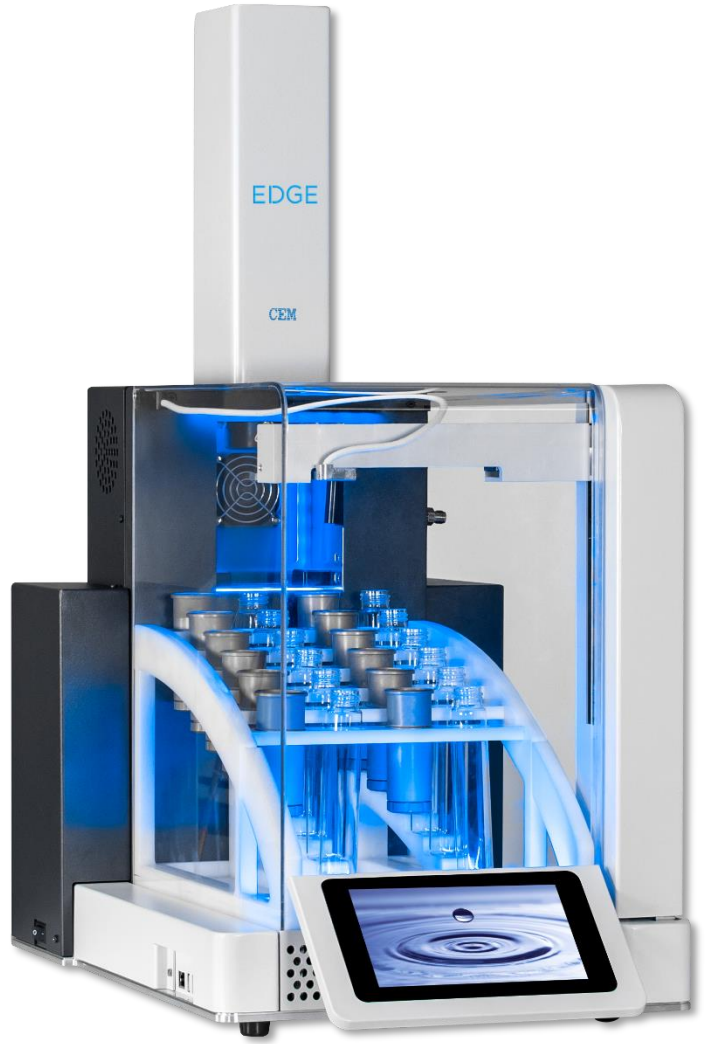




Automated extraction: method optimization

Method (AMXX)	Solvent	Volume (mL)	Bubbling time (s)	Hold time (s)	T (º 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**	**-**

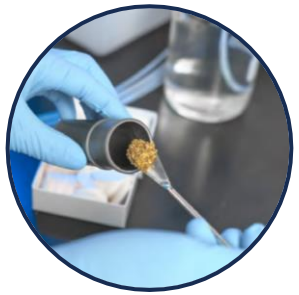


- AcN was the most efficient solvent
- Bubbling (agytation) was deemed counterproductive
- A rinse step significantly improved recovery values

EDGE instrument and pictures courtesy of CEM (Charlotte, North Carolina, United States of America)

Cocoa and coffee: extraction & GC analysis

1

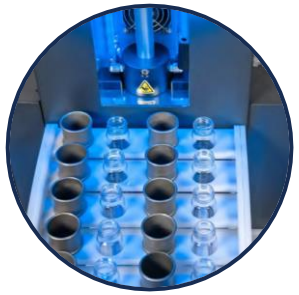


4 g sample

Place filters (Q-Disc) in tube

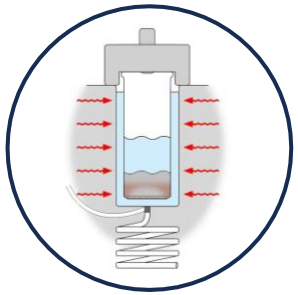
Cover sample with sand or Q-Screen

2



10 mL AcN addition

3

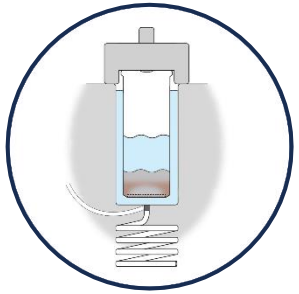


Hold 150 s at 40 ºC

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

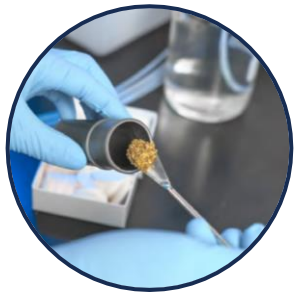
GC-MS/MS Analysis

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.

EDGE instrument and pictures courtesy of CEM (Charlotte, North Carolina, United States of America)

Cocoa and coffee: extraction & LC analysis

1

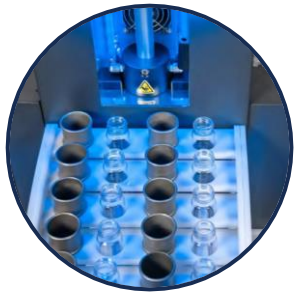


4 g sample

Place filters (Q-Disc) in tube

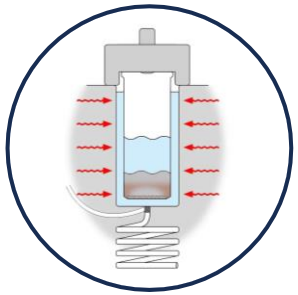
Cover sample with sand or Q-Screen

2



10 mL AcN addition

3

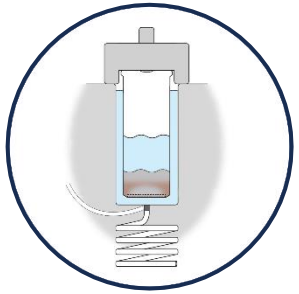


Hold 150 s at 40 ºC

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

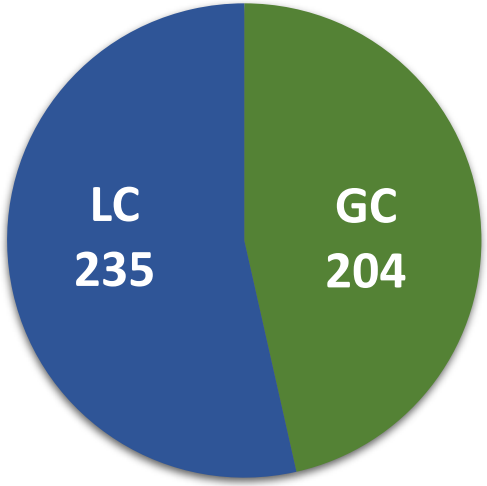
LC-MS/MS Analysis

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.

EDGE instrument and pictures courtesy of CEM (Charlotte, North Carolina, United States of America)

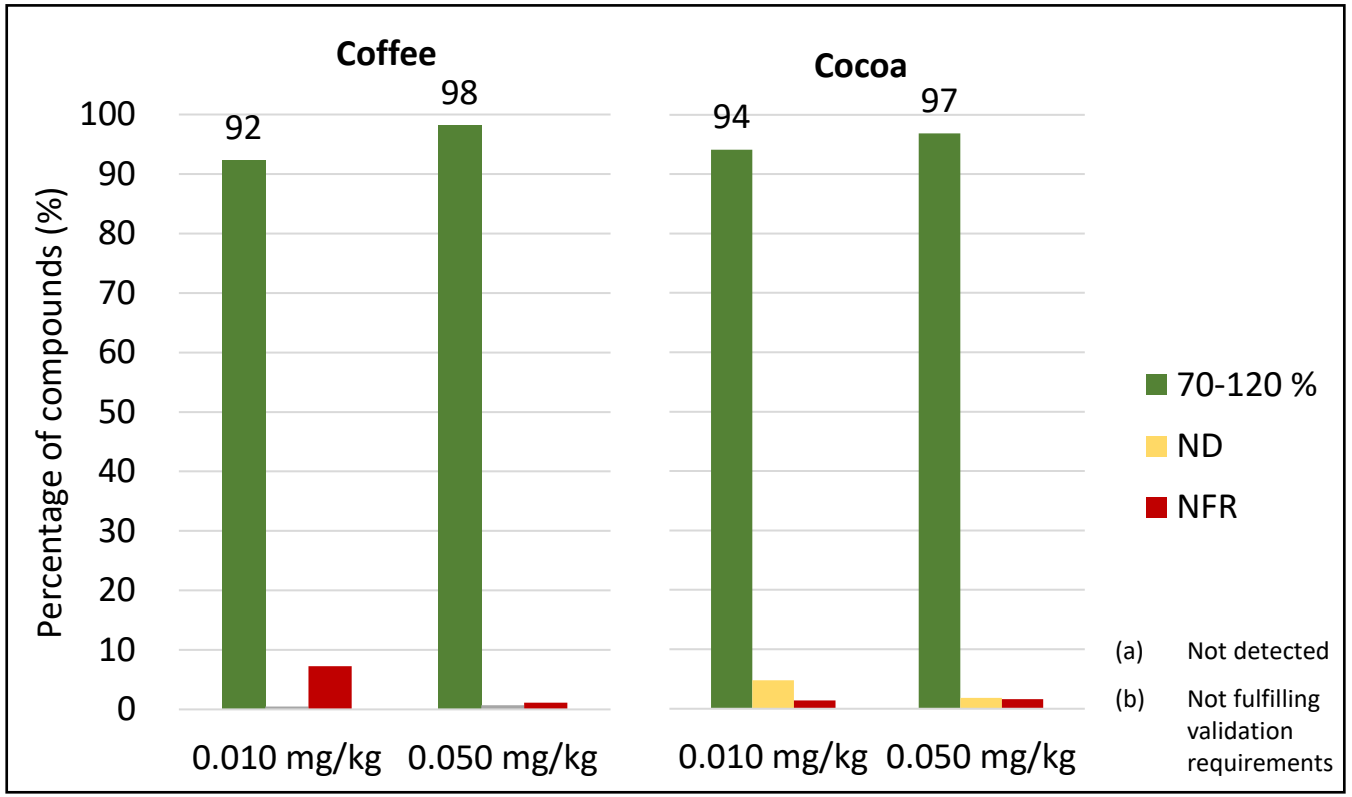
Cocoa and coffee: pesticide residues evaluated

- **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



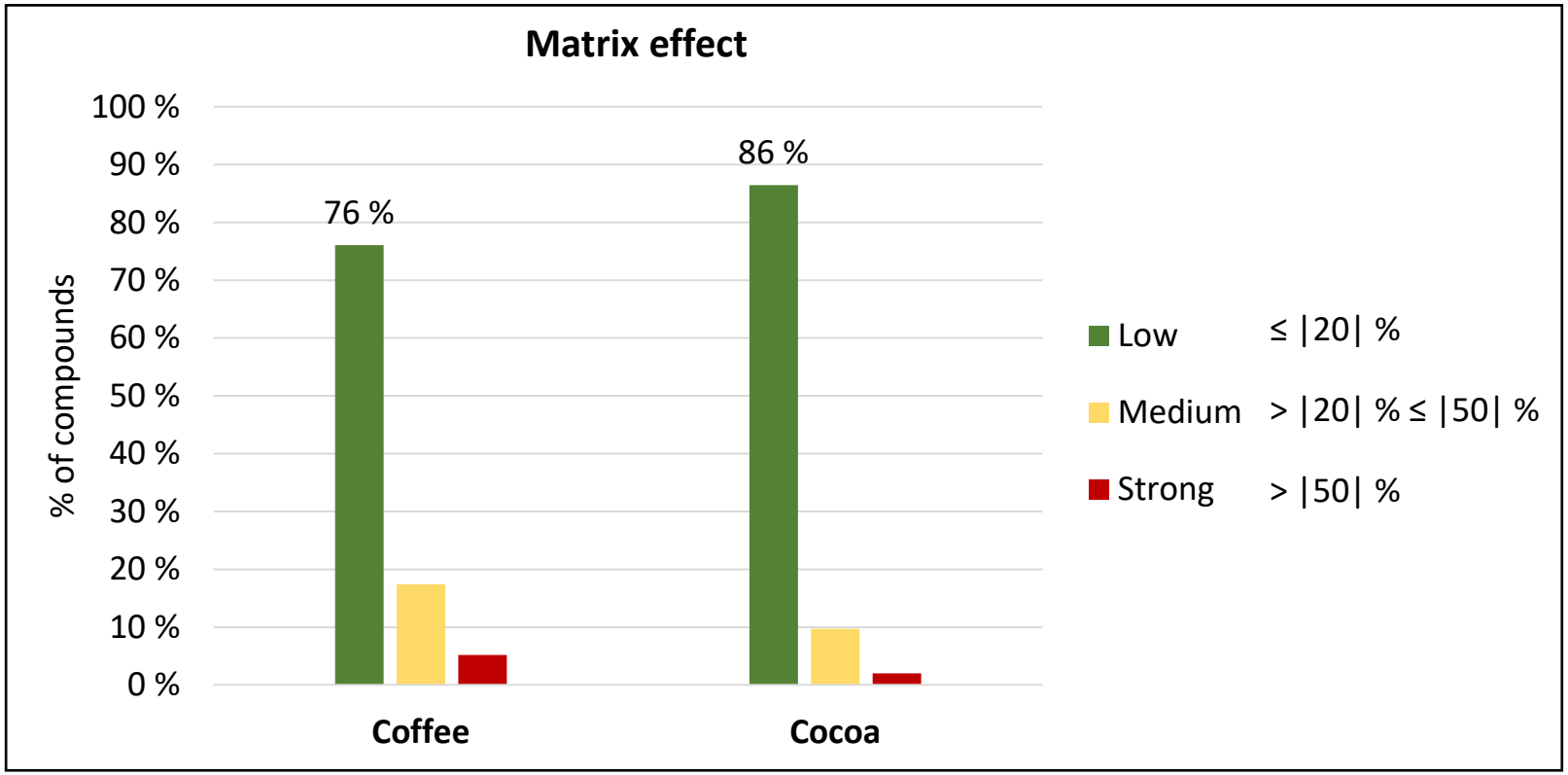
Cocoa and coffee: results for the automated method

- Over 90 % of compounds successfully validated at 0.01 mg/kg with $RSD_r \leq 20$ %



Cocoa and coffee: results for the automated method

- 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



Manual extraction

- Sample hydration causes the coextraction of matrix components that hinder the analysis

2 g sample
+
4 mL H_2O (*wait 30 min*)
+
10 mL AcN
1

Shake 7 min

4 g $MgSO_4$, 1 g NaCl, 1 g $Na_3Citrate{\cdot}2\ H_2O$, 0.5 g $Na_2HCitrate{\cdot}1.5\ H_2O$
2

Shake 7 min
Centrifuge 5 min

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

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

Vortex 30 s
Centrifuge 5 min

ANALYSIS
5

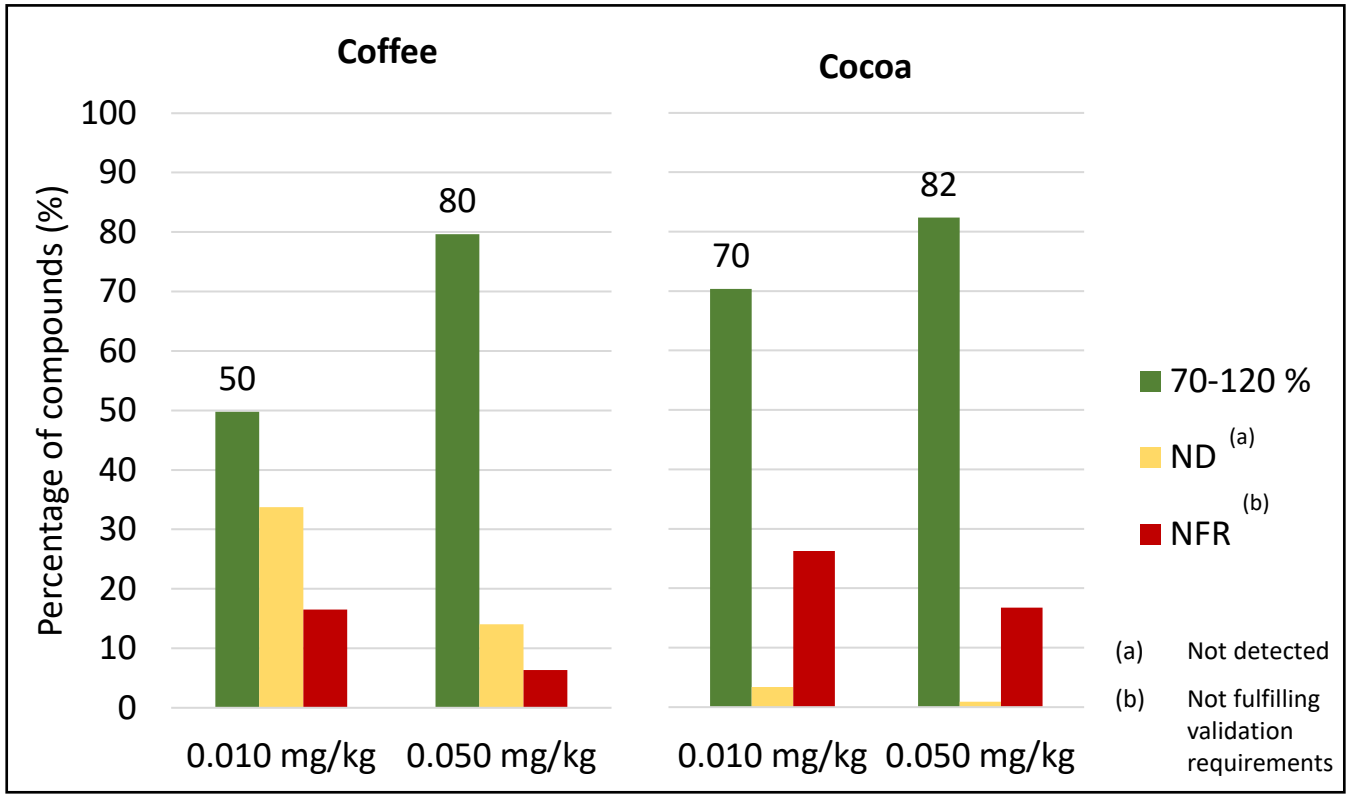
GC-MS/MS
Evaporate 50 µL of the extract and dissolve in AcOEt

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.

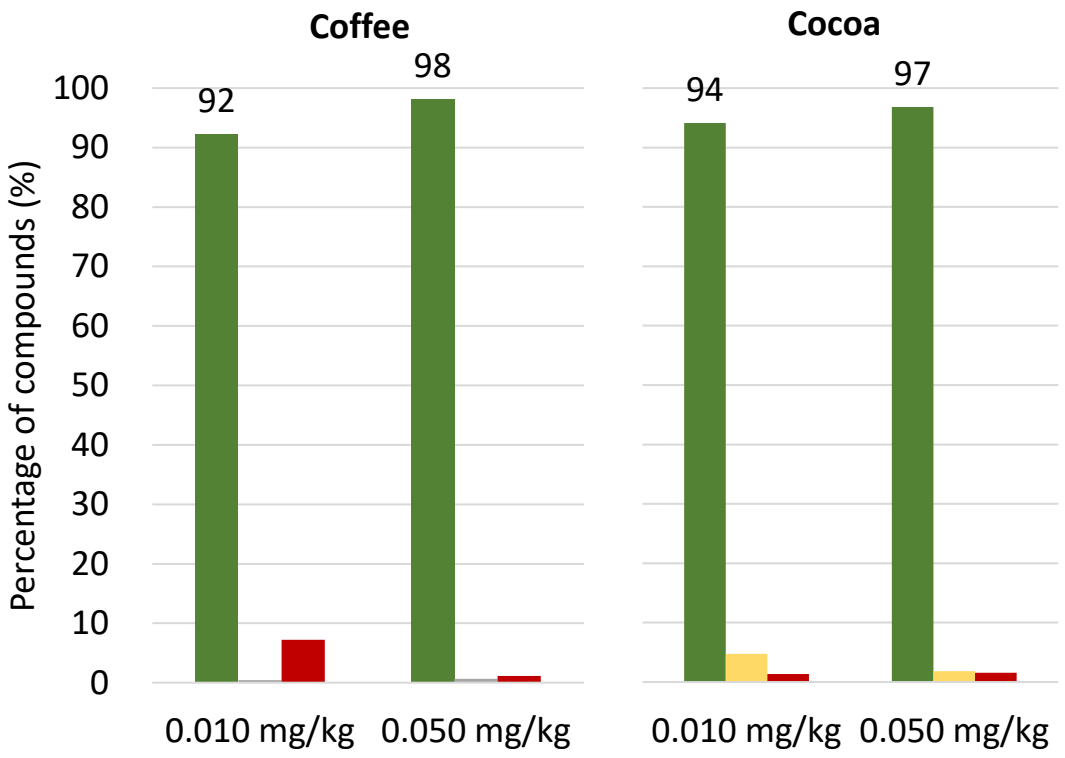
Cocoa and coffee: results for the manual method

- Far fewer compounds could be successfully validated with this method. Worth noting the high number of non-detections in the case of coffee

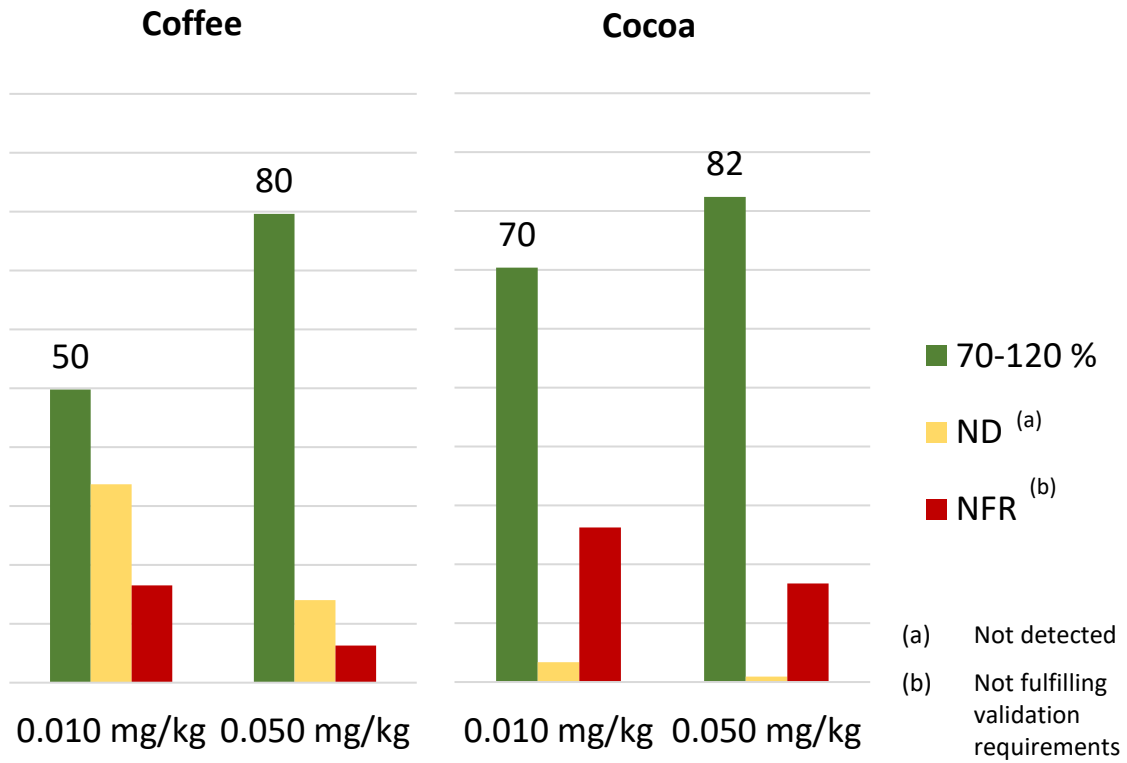


Comparison between extraction methods

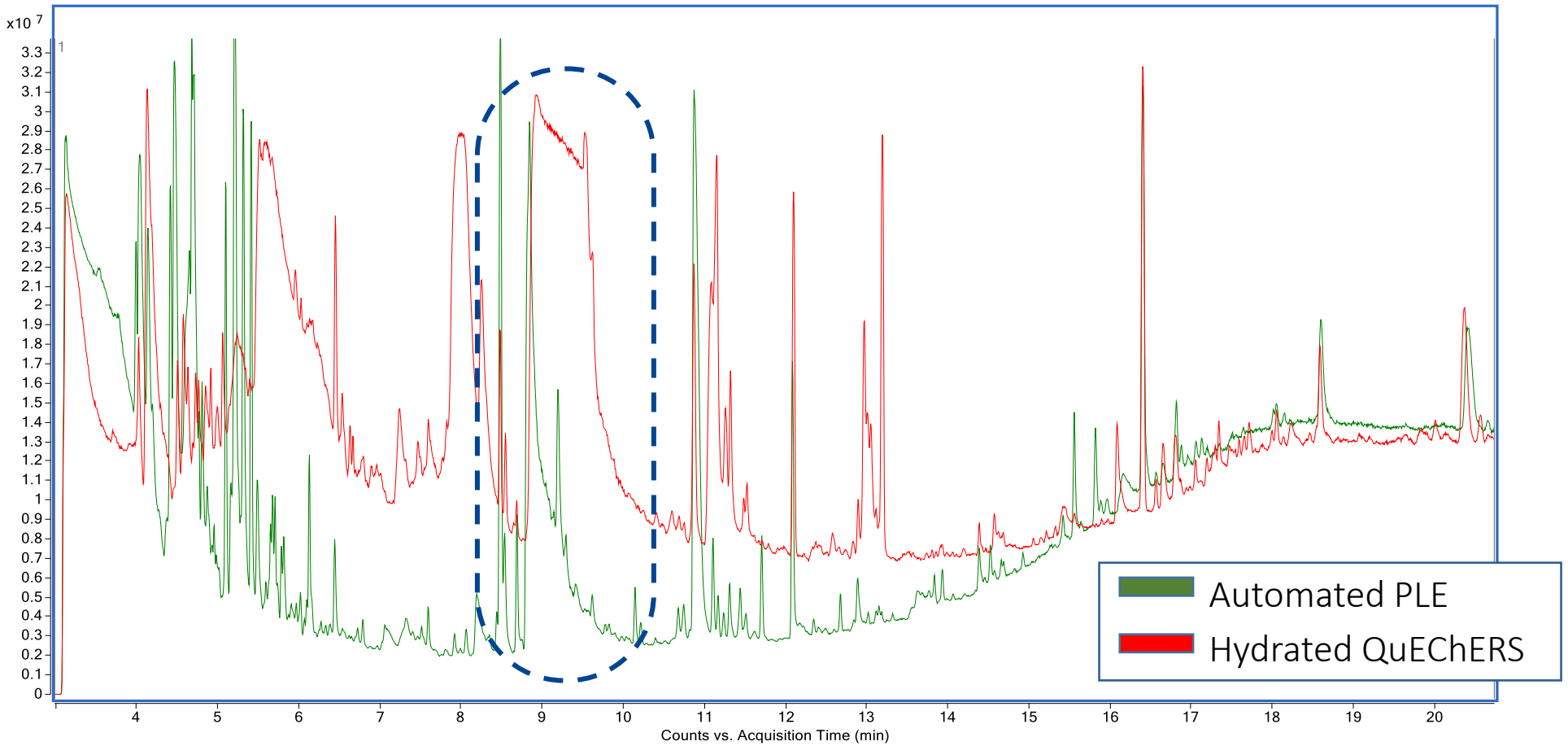
Automated extraction
(*Pressurized liquid extraction*)



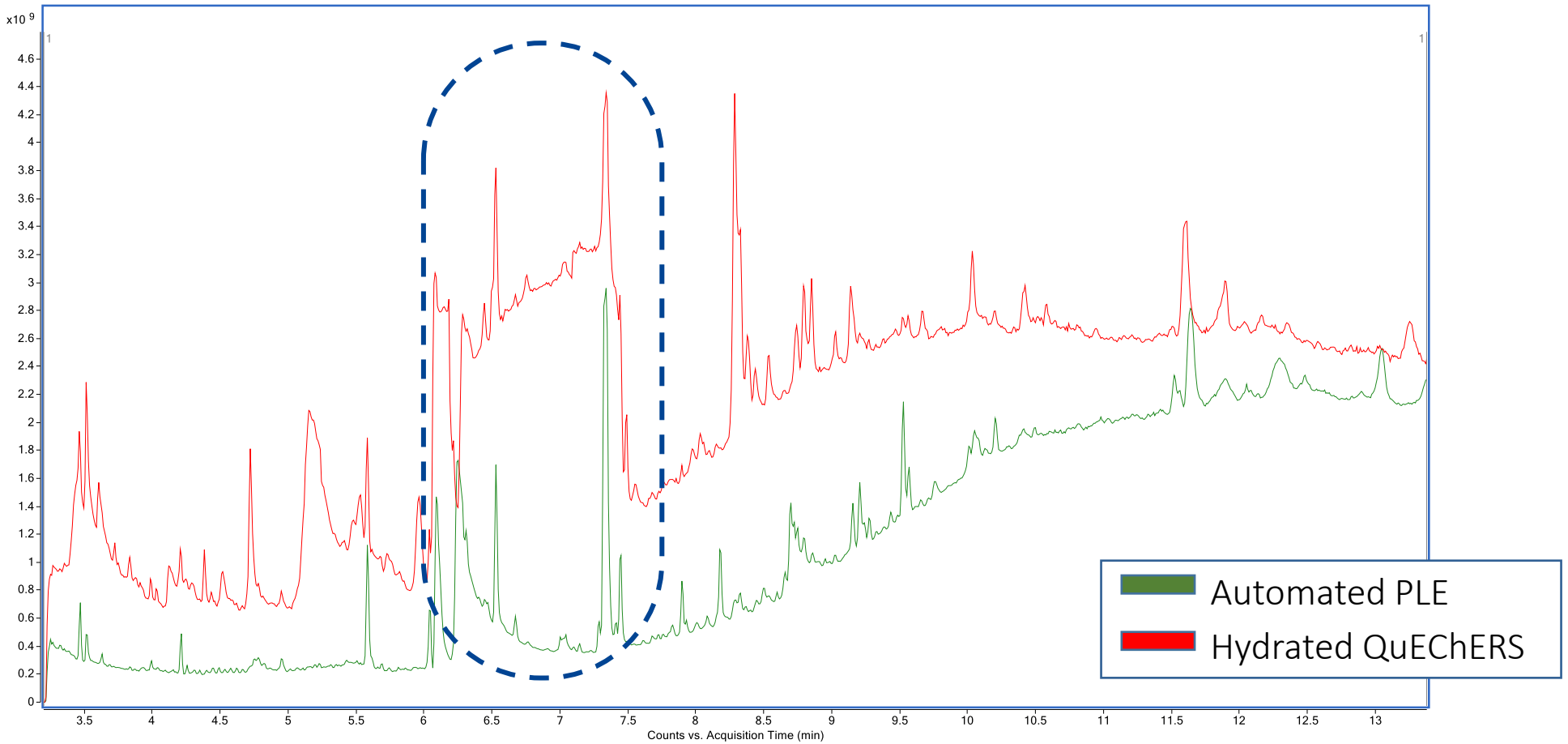
Manual extraction
(QuEChERS with hydration)



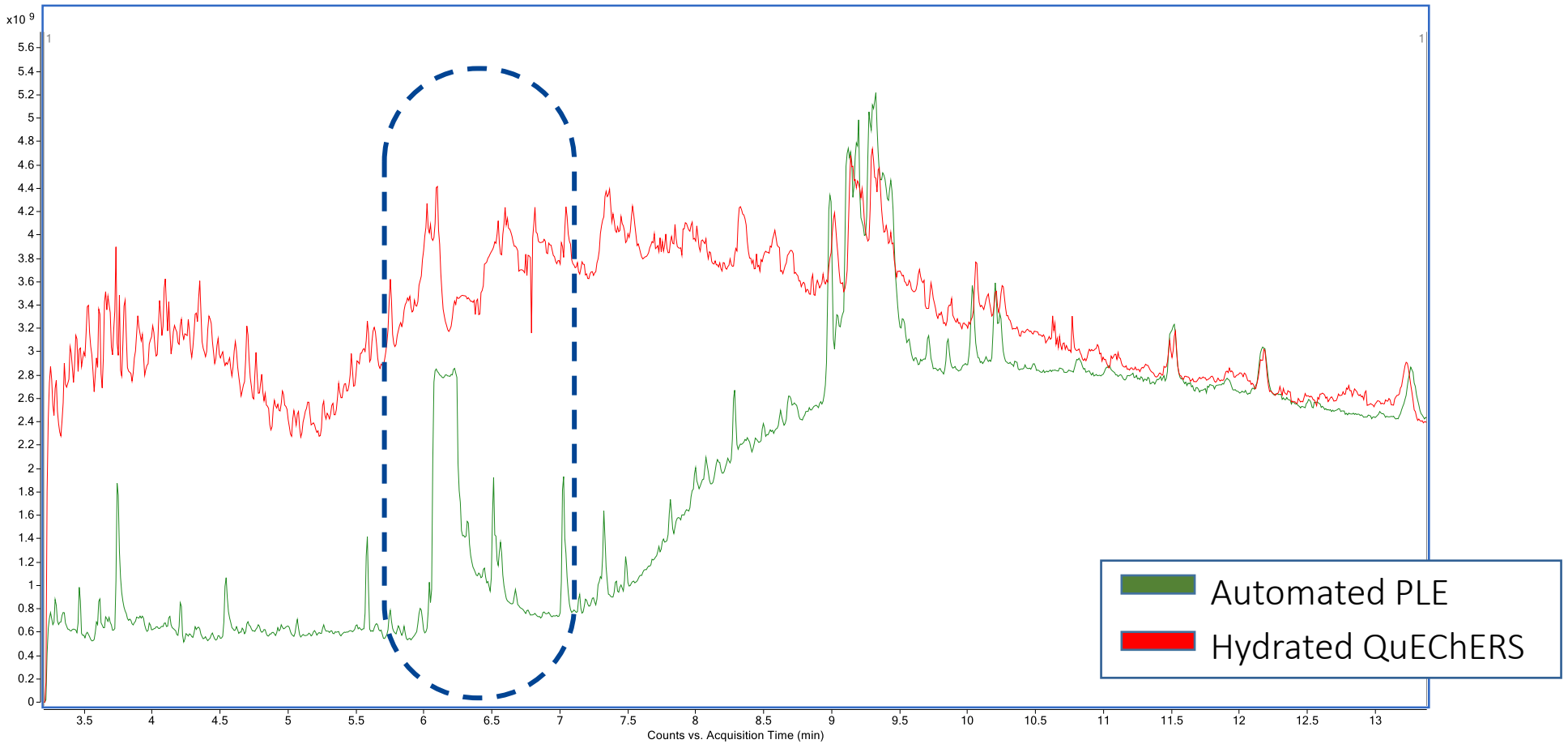
Total ion chromatogram of tea



Total ion chromatogram of cocoa

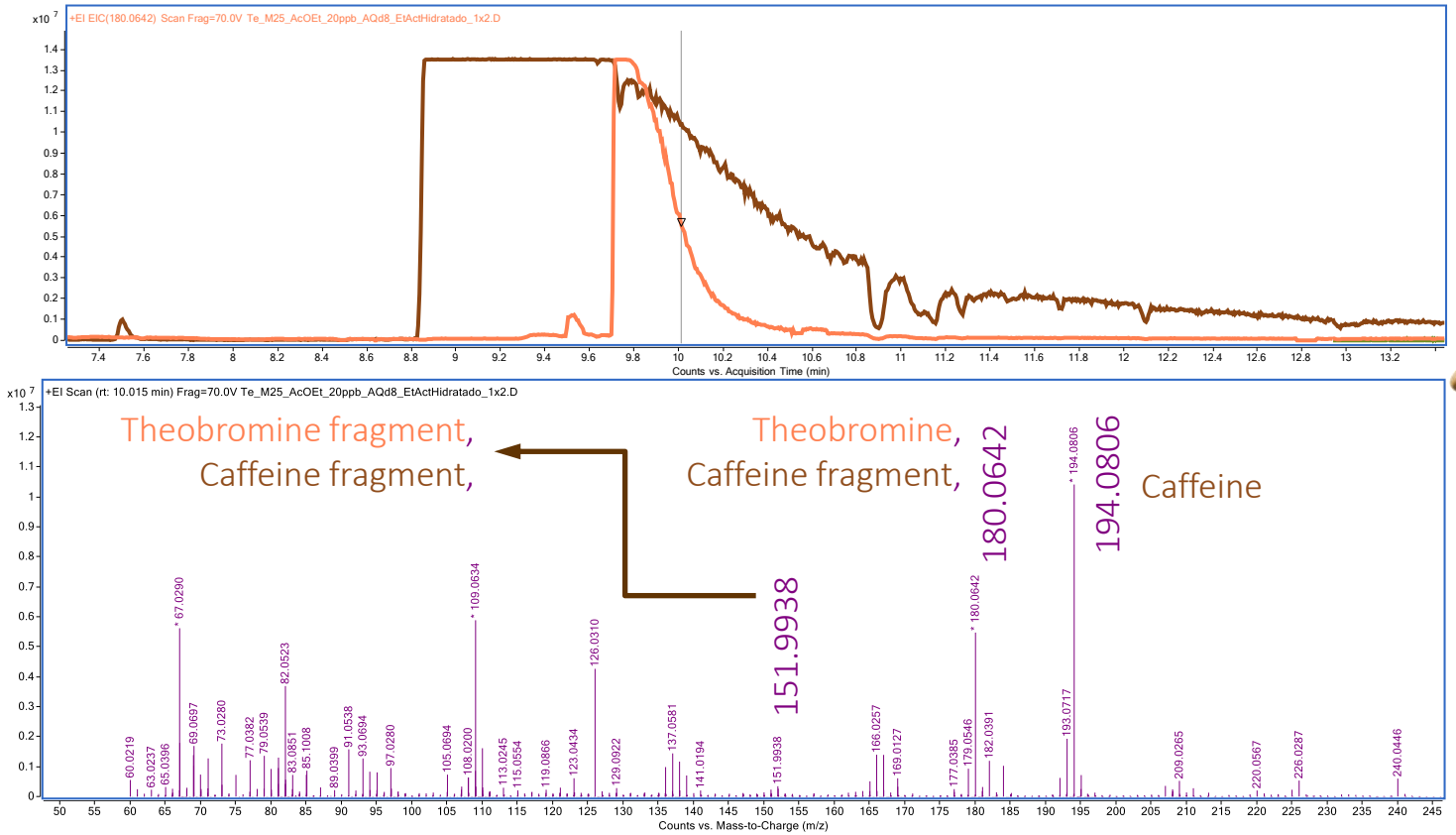


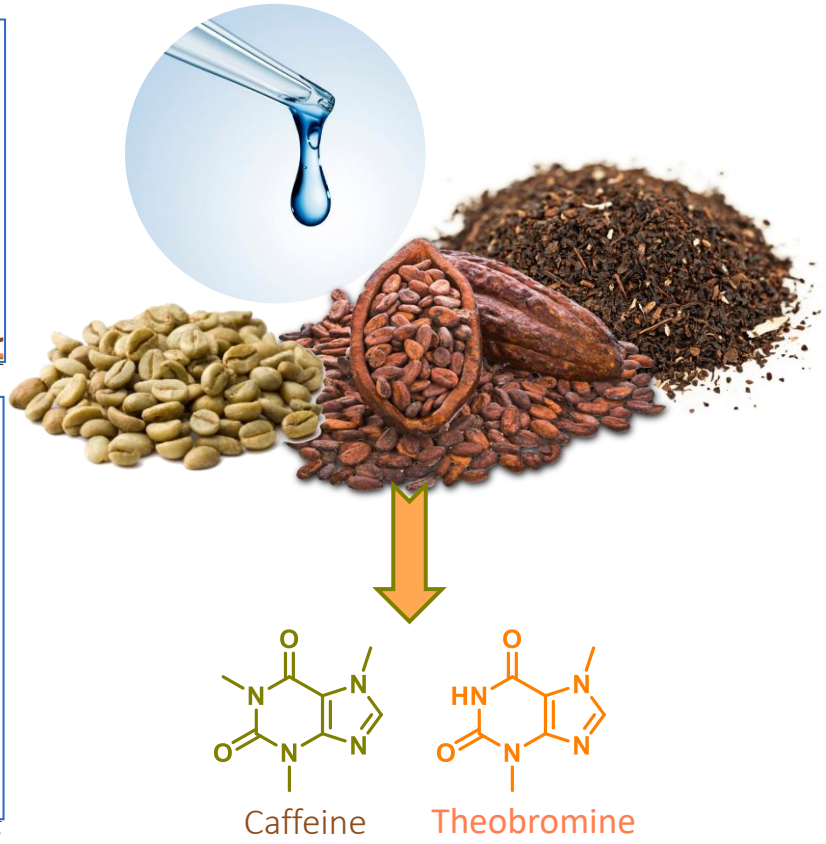
Total ion chromatogram of coffee



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

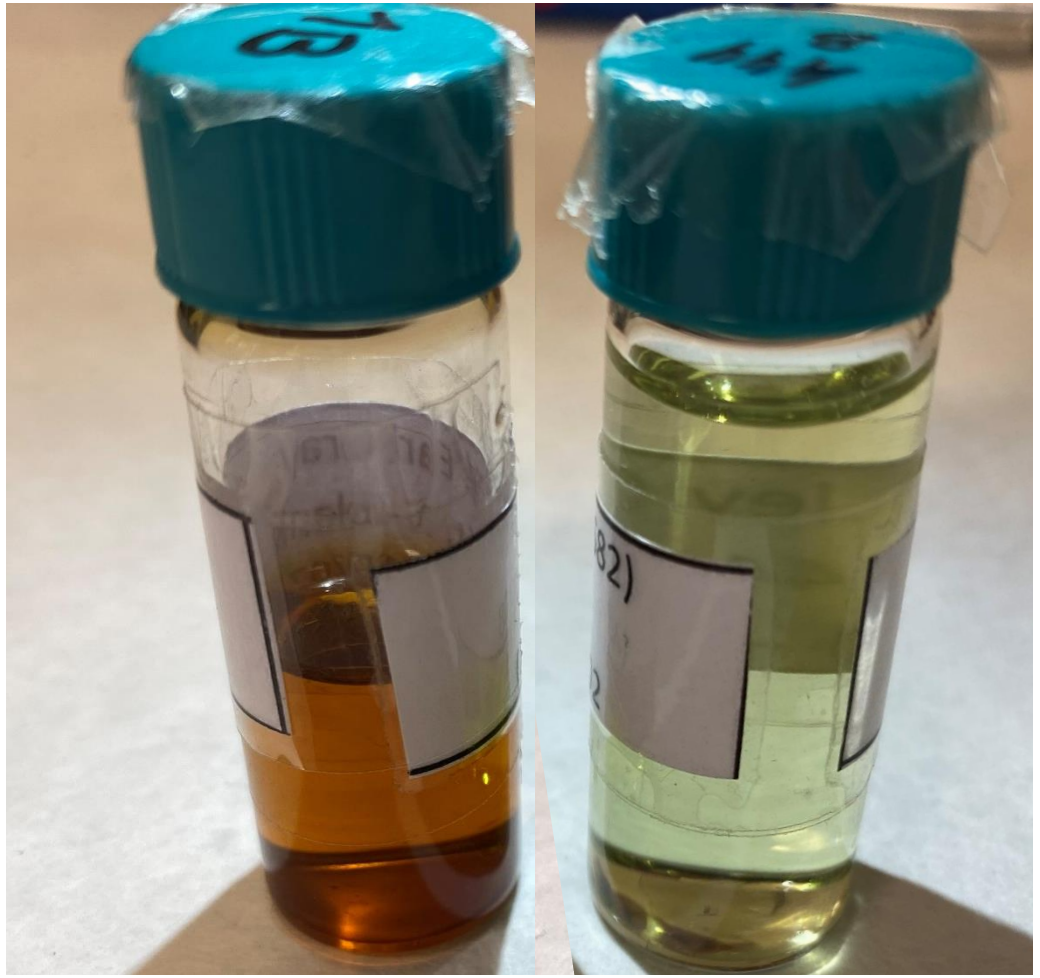




Final acetonitrile extracts visual comparison in tea

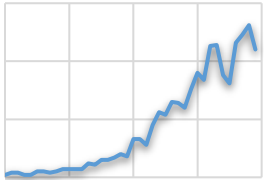
Manual extraction

Automated PLE extraction (EDGE)



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

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 inert gas
- Thorough traceability: *who* ran the sample, *when* was the sample run, *what* were the extraction conditions, and possibility to export all the data to a computer

Future work

- Extend the method to other fruits, and vegetables (all matrix groups)

- Extend the method to other pesticides, such as highly-polar pesticides

- Develop new methods for matrixes or analites not fit for the current one

References

- EURL-FV (2019-M34) Development and validation of a Multiresidue Method for high fat content commodities: coffee and cocoa beans
  - https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?LabID=500&CntID=828&Theme_ID=1&Pdf=False&Lang=EN

- 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.


Pre-COVID-19 picture


Thank you for
your attention