NEW ADVANCES IN THE ANALYSIS OF MRM COMPOUNDS

IMPLEMENTATION OF AUTOMATED SAMPLE EXTRACTION USING PRESSURIZED LIQUID TECHNOLOGY FOR DIFFICULT MATRICES.

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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.*

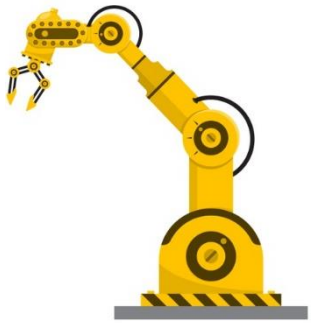
- Energetic extraction conditions must be employed if no sample hydration is to be employed

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

Solution?

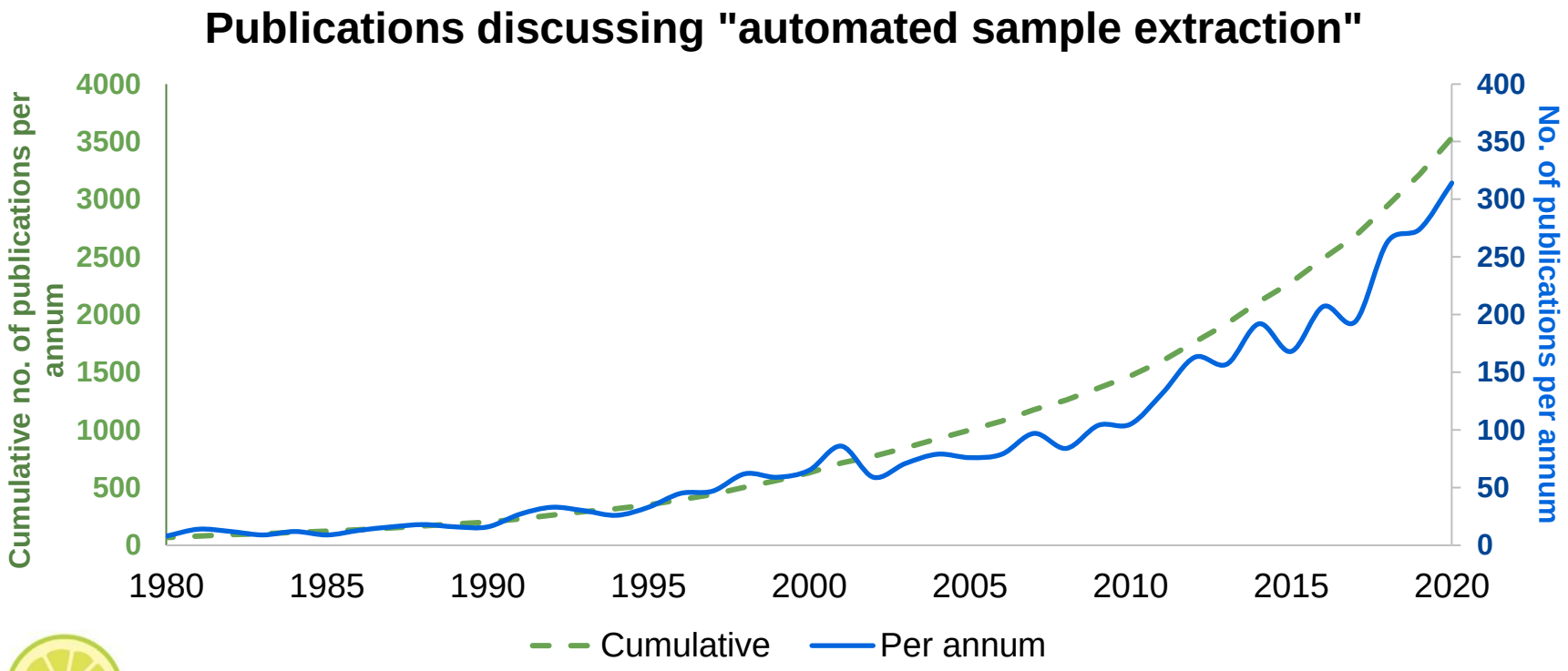
High energetic extraction with organic solvents

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



Commercially available instrumentation

ANKOM FLEX
Analyte Extractor

CEM EDGE®
Automated Solvent
Extraction System

FMS PLE®
And SuperVap®
Concentrator

Dionex ASE®
Accelerated
Solvent Extraction

Automated extraction

Difficult matrices

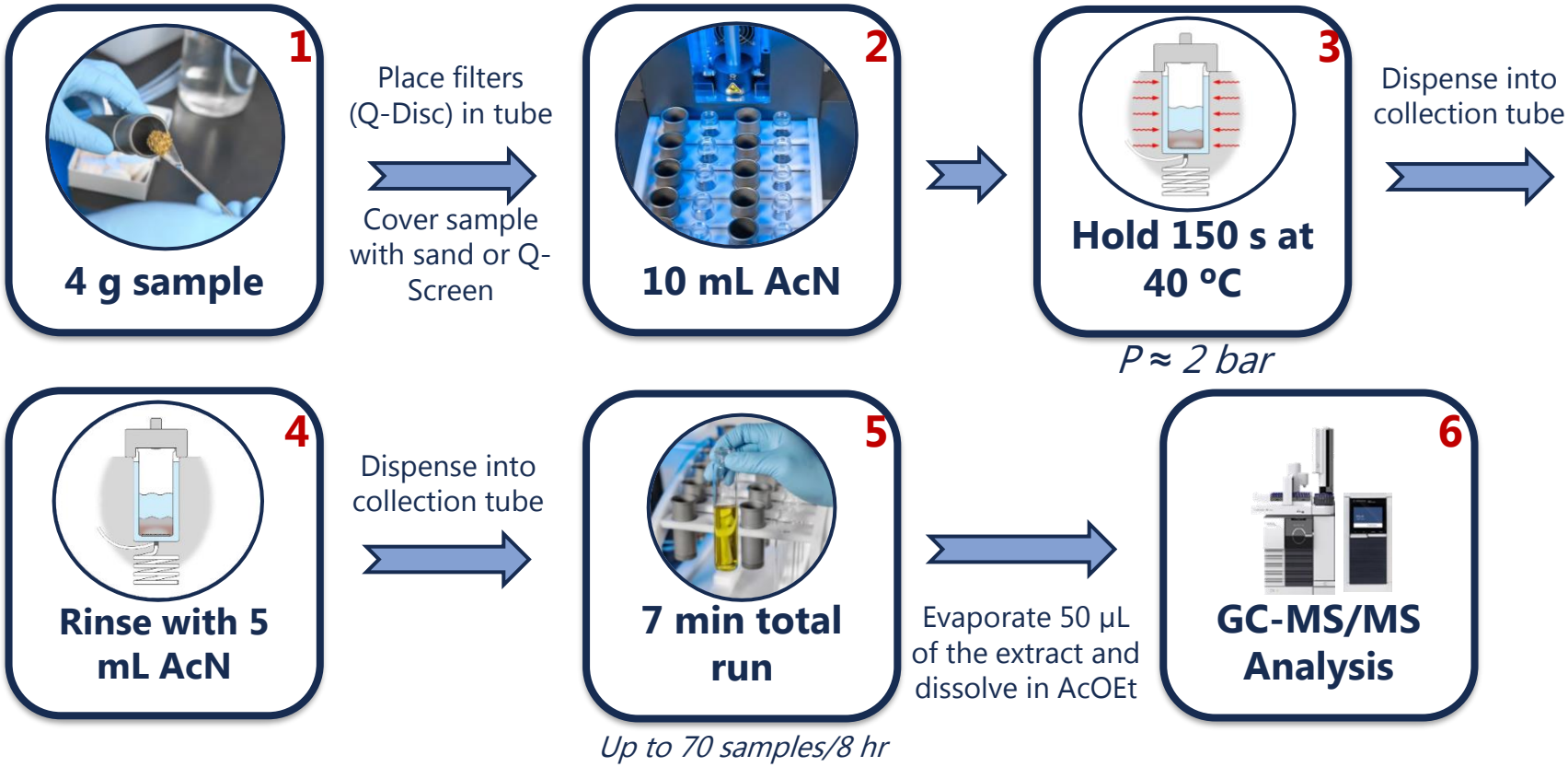
Tea, coffee beans & cocoa beans

Automated extraction: method optimisation

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**	**-**

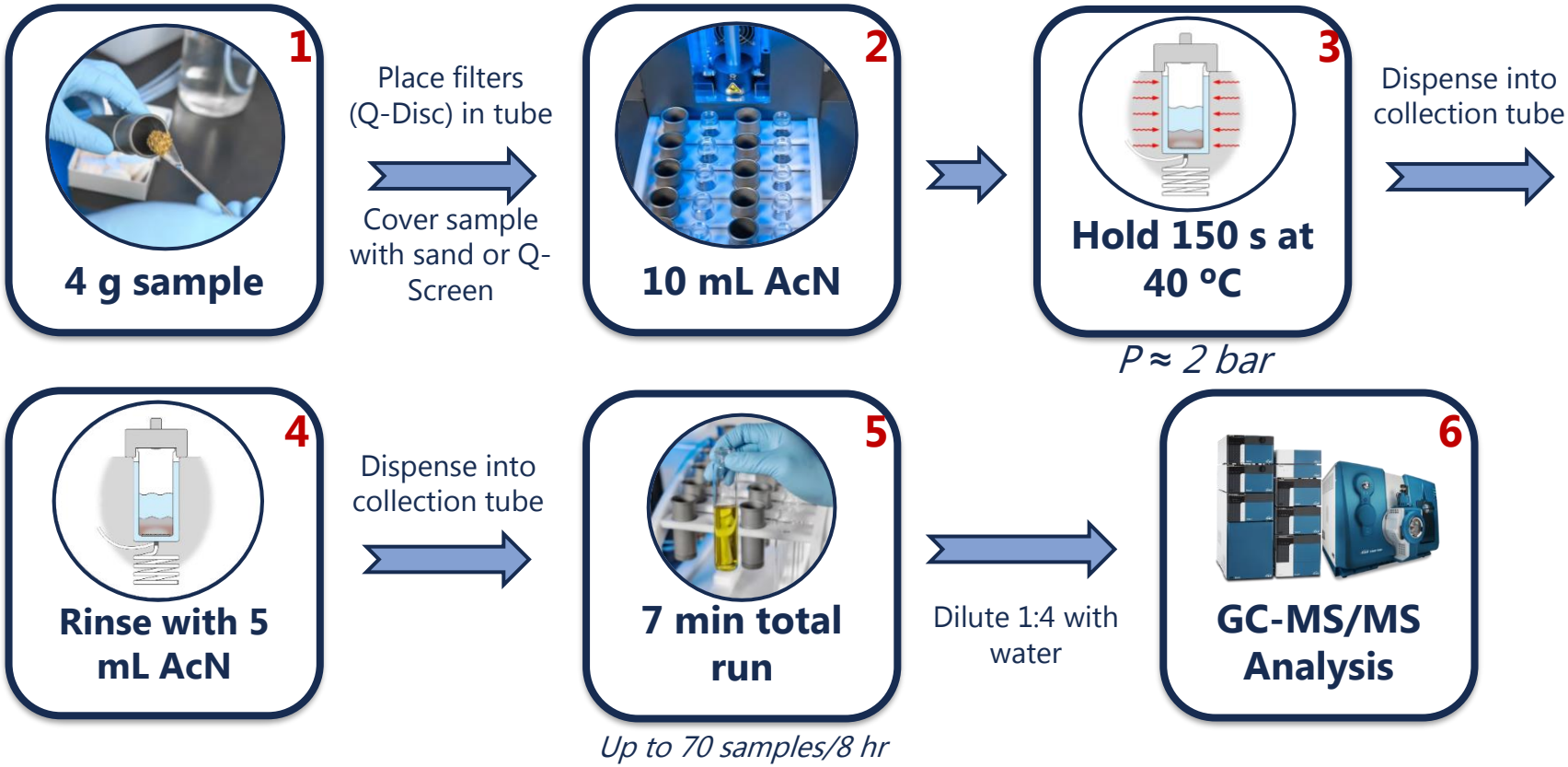
- AcN was the most efficient solvent
- Bubbling (agitation) with air was deemed counterproductive
- A rinse step significantly improved recovery values

Cocoa and coffee: extraction and GC 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 and LC analysis



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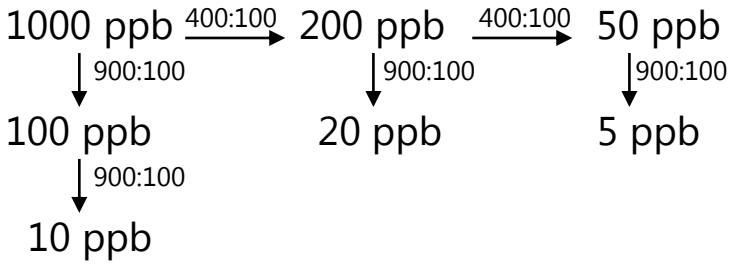
Cocoa and coffee: vials injection (LC-MS/MS)

- **After extraction** ⟶ 4 grams/ 15 mL = **3,75 dilution**
  - Mix peticides 1000 ppb/ 3,75 = 267 ppb ⟶ simulate that 267 ppb is in reality 1000 ppb

- Solvent calibration curve in AcN at 200, 100, 50, 20, 10 and 5ppb



- Solvent calibration curve (**dilution 5**)
  - 200 ppb (x5): 400 µL H_2O + 100 µL mix 200 ppb AcN
  - 100 ppb (x5): 400 µL H_2O + 100 µL mix 100 ppb AcN
  - …

- Recovery, samples or blank (**dilution 5**)
  - 400 µL H_2O + 100 µL extract

- Matrix-matched calibration curve (**dilution 5**)
  - 200 ppb (matrix, x5): 100 µL blank matrix ⟶ (evaporate) 100 µL Mix 200 ppb ⟶ (vortex) 400 µL H_2O
  - 100 ppb (matrix, x5): 100 µL blank matrix ⟶ (evaporate) 100 µL Mix 100 ppb ⟶ (vortex) 400 µL H_2O
  - …

Manual extraction (QuEChERS): coffee, cocoa and tea

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

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

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**
3 mL extract
+ 150 mg $CaCl_2$
+ 150 mg PSA

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.

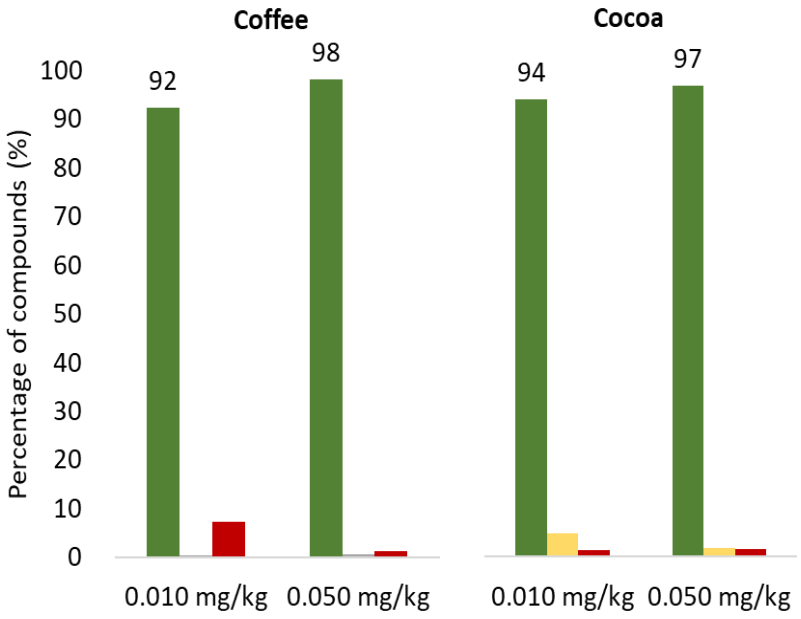
Cocoa and coffee: pesticide residues evaluated

- **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

Comparison between extraction methods

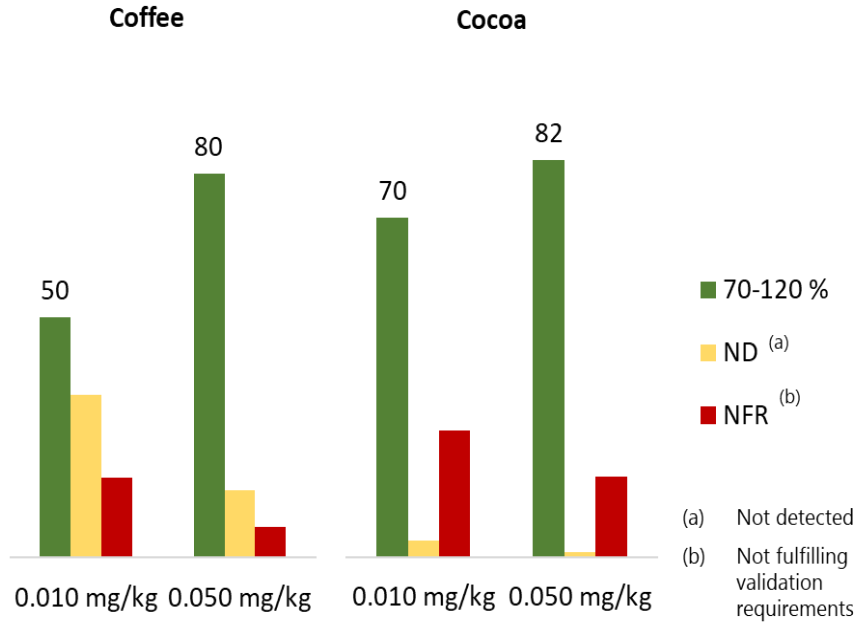
Automated extraction
(*Pressurized liquid extraction*)

Over 90 % of compounds successfully validated at 0.010 mg/kg with $RSD_r \leq 20$ %



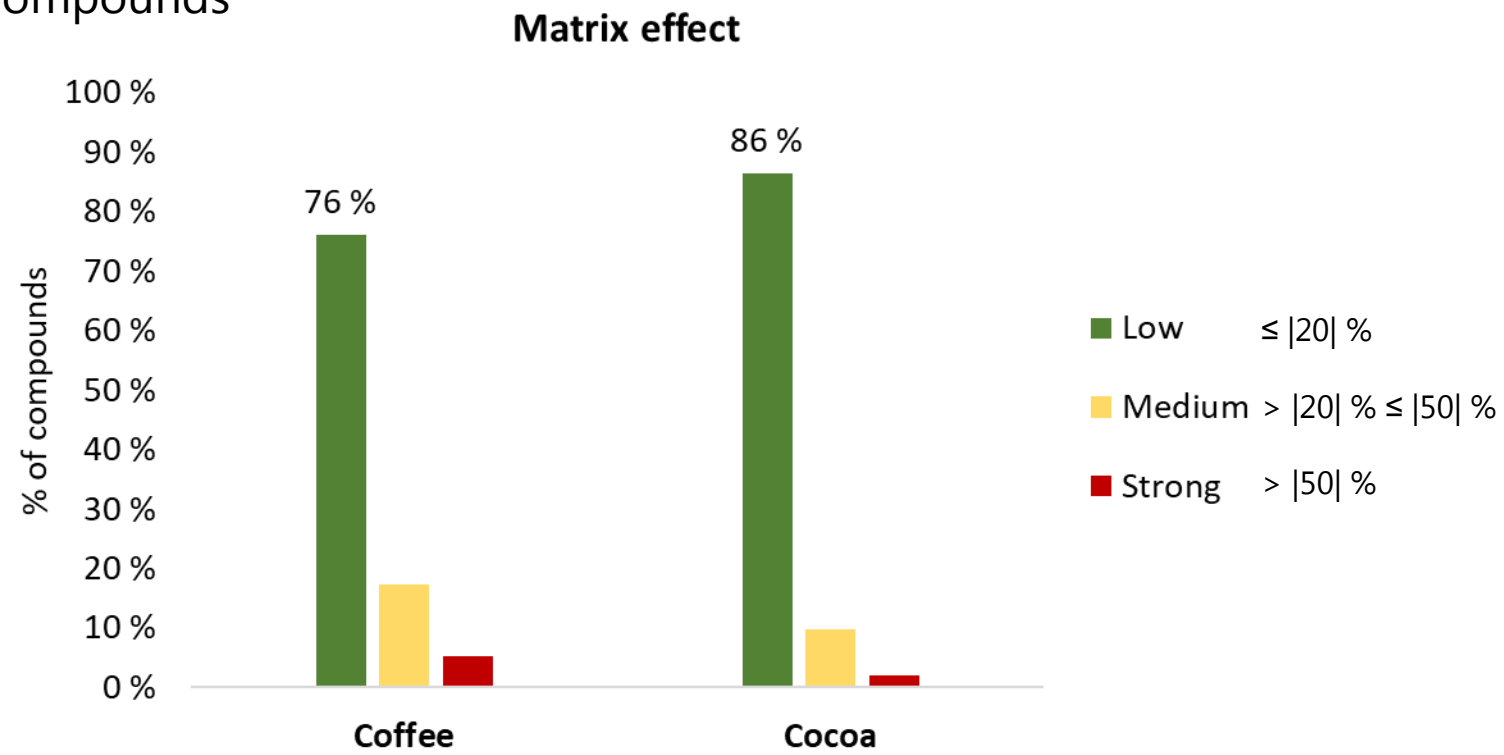
Manual extraction
(QuEChERS with hydration)

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

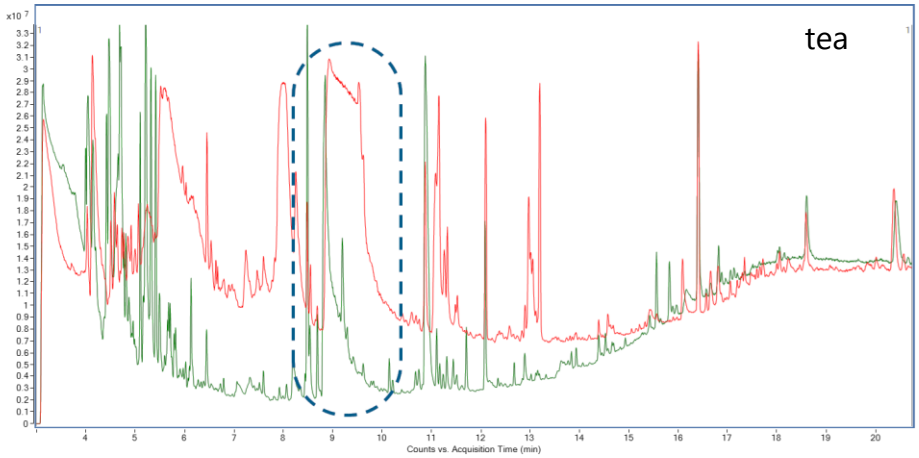


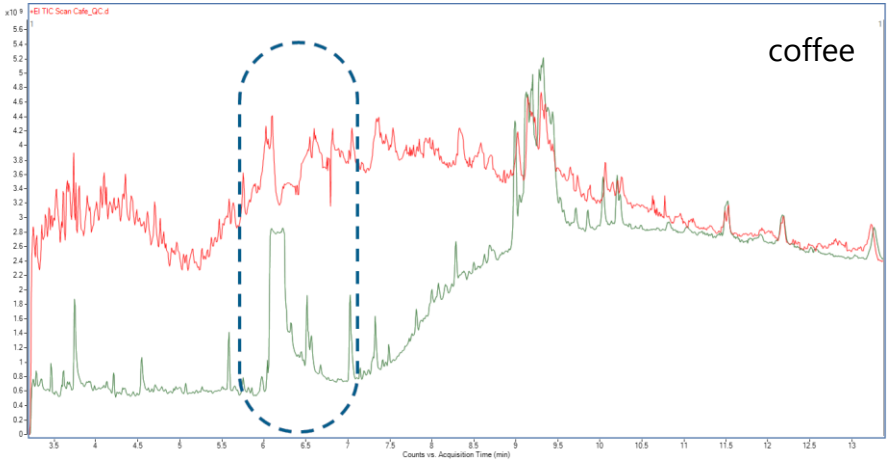
Automated method matrix effect

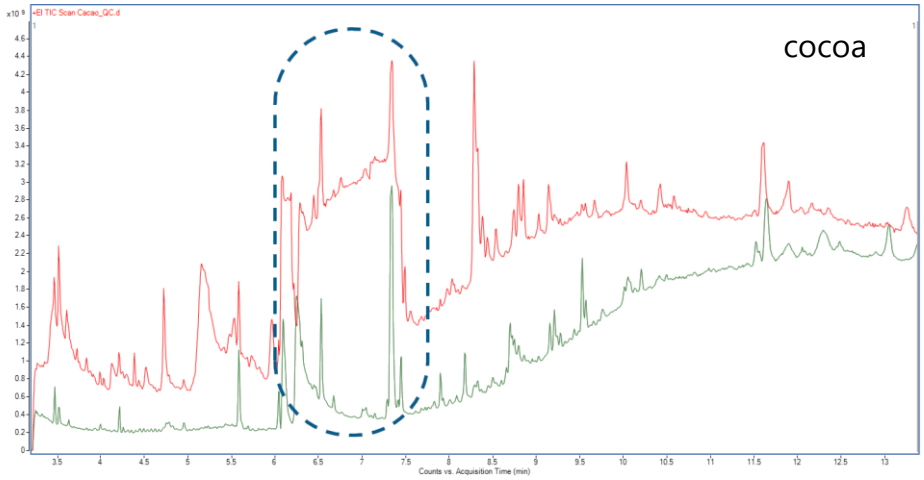
- Linearity and matrix effect were evaluated in the 0.005 – 0.200 mg/L range
  - Correlation coefficient was ≥ 0.99 in all successfully validated compounds



Total ion chromatogram



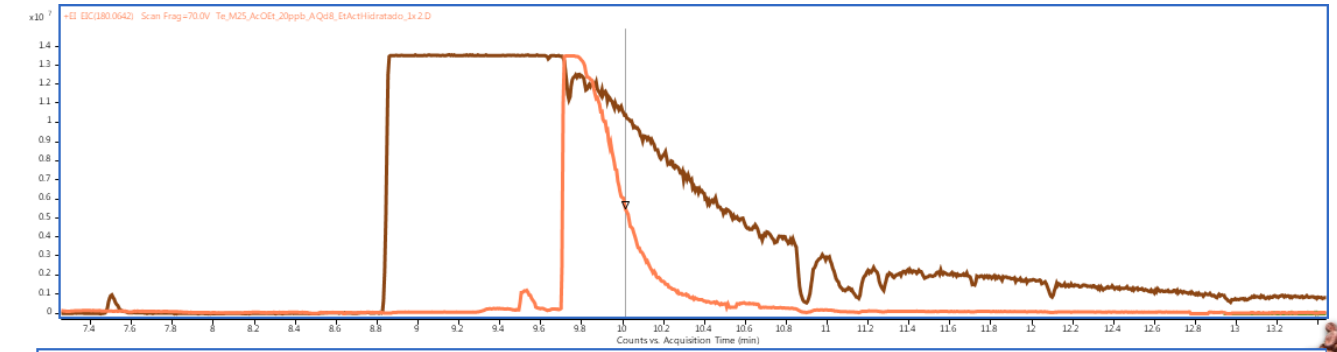


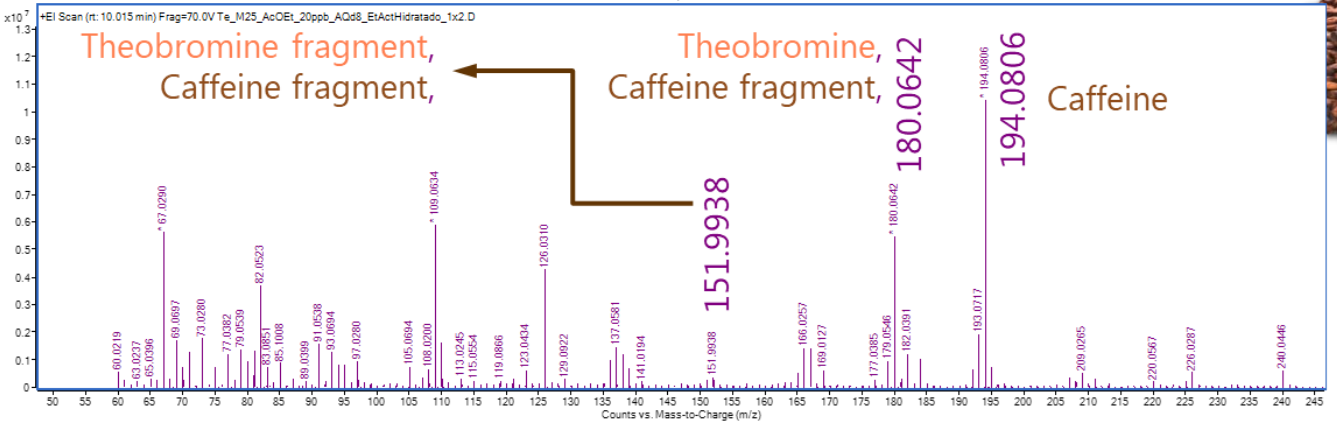


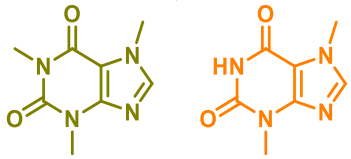
Automated PLE
Hydrated QuEChERS

Main interferences in hydrated methods

- Caffeine and theobromine have been identified as the main coextracted matrix interferences using an Agilent 7250 GC/Q-TOF HRAMS instrument

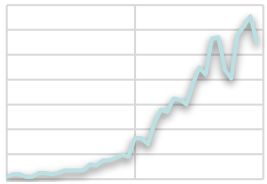






Caffeine Theobromine

Conclusions



- Automation in laboratories is a key objective in routine analysis

- Pressurized liquid extraction is a viable alternative for sample extraction of matrices 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

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

Conclusions: advantage of PLE (EDGE)

- Replaces tedious, manual extraction procedures with difficulties in reproducibility

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

- Possibility of “bubbling” with an inert gas such as nitrogen

- 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 water 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.

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Thank You
for Your Attention