Analysis of dry commodities using pressurized sample extraction to overcome the issues associated with sample hydration

Díaz-Galiano, Francisco José

Murcia-Morales, M.; Gómez-Ramos, M. M.; Beraza, I.; Fernández-Alba, A. R.*

diaz-galiano@ual.es; amadeo@ual.es*

1

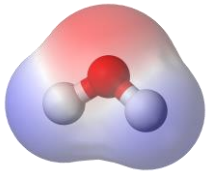
Automated extraction of tea

Method development

Sample hydration: pros and cons

- SANTE Document recommends sample hydration prior to extraction

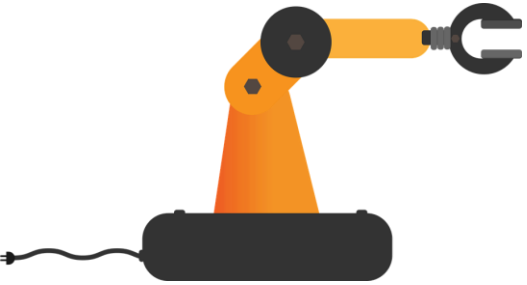
- Sample hydration increases extraction of polar compounds, but may hinder the extraction of certain apolar compounds



- 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
- 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



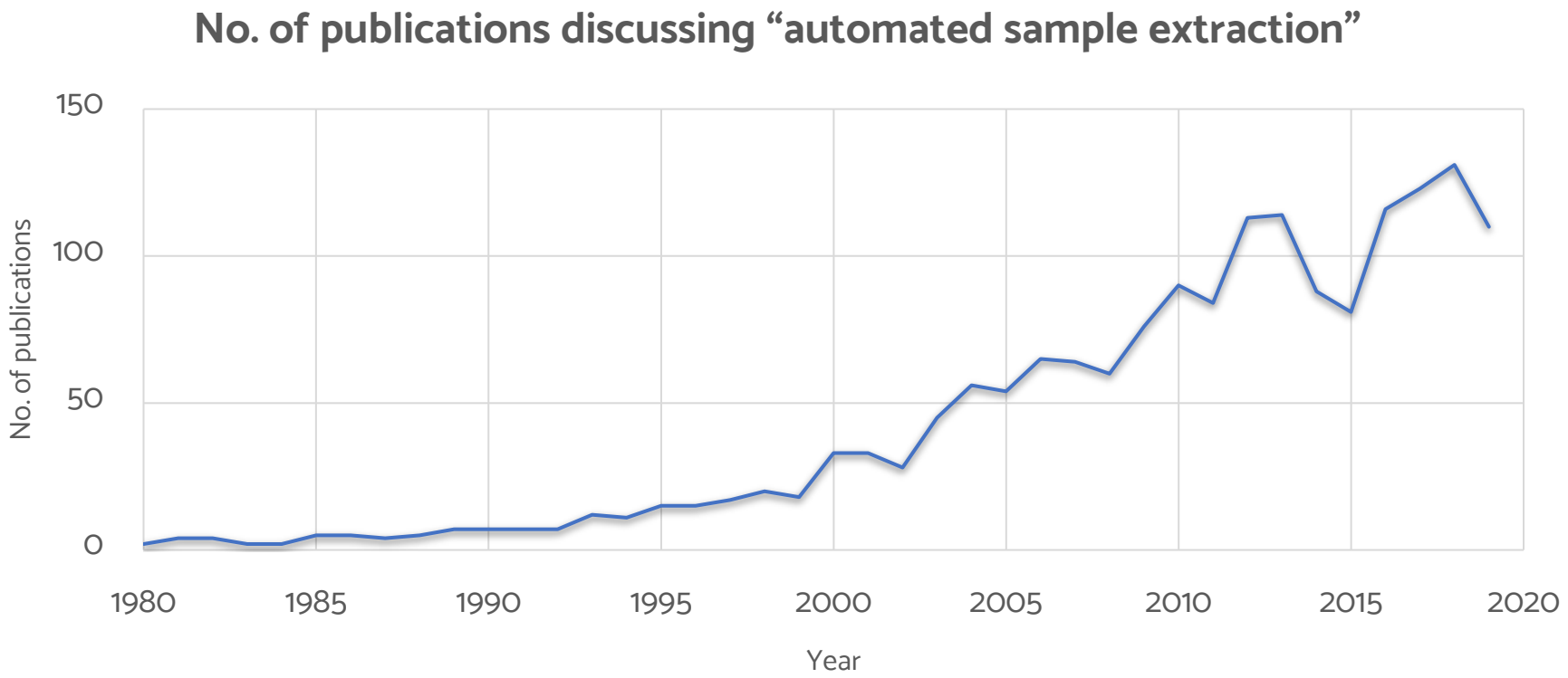
Solution?

Automated sample extraction

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 personnel cost reduction



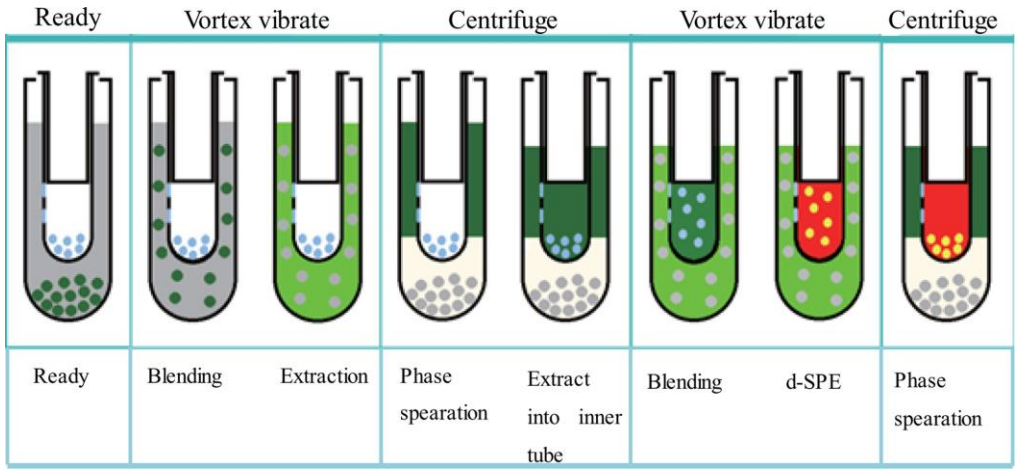
Search on Scopus with the terms “‘automated extraction” OR “automated sample extraction”’

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

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

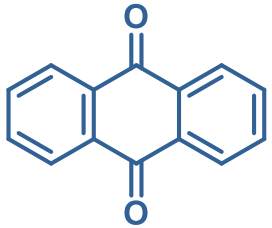


J. Wang, Z. He, L. Wang, Y. Xu, Y. Peng, X. Liu, Automatic single-step quick, easy, cheap, effective, rugged and safe sample preparation devices for analysis of pesticide residues in foods, J. Chromatogr. A. 1521 (2017) 10–18.

Automated extraction of tea: the case of anthraquinone

EPRW
2020 · GRANADA

- Anthraquinone (AQ) is an aromatic organic compound linked to adverse health effects
- The current MRL is set at 0.02 mg/kg in tea
- AQ was first reported by EFSA in tea in 2012
- Since 2012, 52 notices have been issued for this commodity, up to 0.36 mg/kg



Automated extraction of tea: problems with manual extraction

EPRW 2020 · GRANADA

- Tea hydration causes the coextraction of matrix components that hinder the analysis of AQ

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

→ Shake 7 min

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

→ 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
→
Evaporate 50 µL of the extract and dissolve in AcOEt

ANALYSIS **5**

GC parameters
Agilent Intuvo 9000 system
2 planar columns
(15 m × 0.25 mm × 0.25 µm)
Splitless injection
Total runtime 12.4 min
Injection volume 1 µL

MS parameters
Agilent 7010B Triple Quad
Ionization mode: EI
Acquisition mode: dMRM

AQ transitions:
208-152; 180-152; 208-180

A. Lozano, Ł. Rajski, N. Belmonte-Valles, A. Uclés, S. Uclés, M. Mezcua, A.R. Fernández-Alba, 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. 1268 (2012) 109–122.

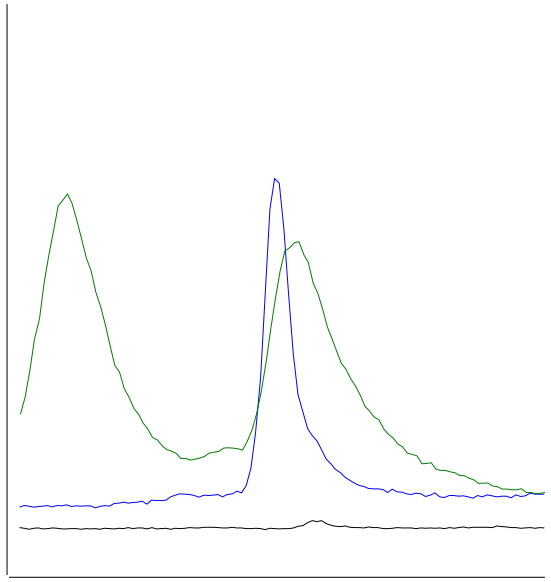
EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

UNIVERSIDAD DE ALMERÍA

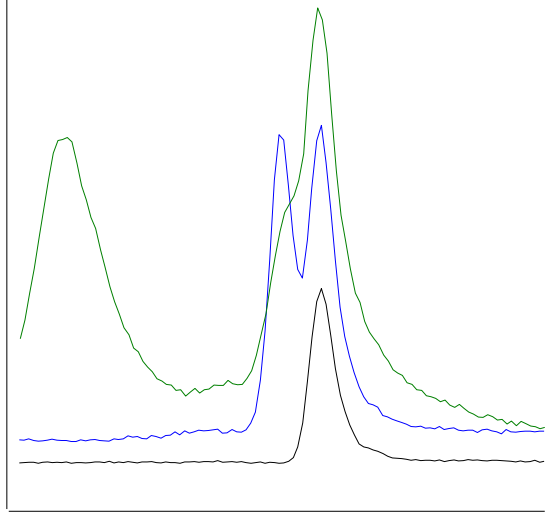
Automated extraction of tea: problems with manual extraction

- Tea hydration causes the coextraction of matrix components that hinder the analysis of AQ

Tea blank

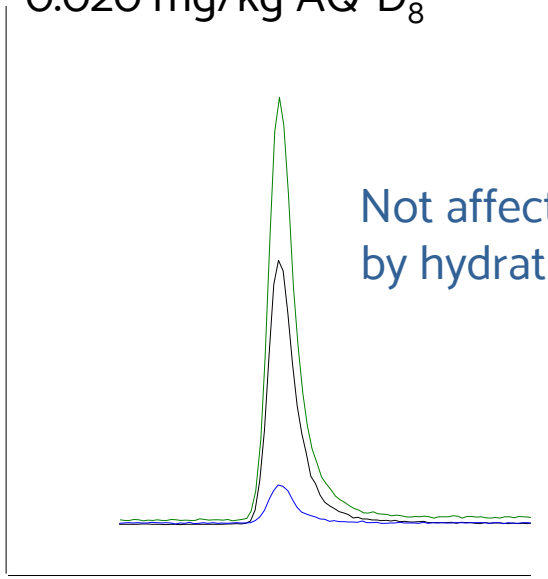


Tea blank spiked with 0.020 mg/kg AQ



208,0 -> 152,0 , 208,0 -> 180,0 , 180,0 -> 152,0

Tea blank spiked with 0.020 mg/kg AQ-D_8



Not affected by hydration

216,0 -> 160,0 , 216,0 -> 188,0 , 188,0 -> 160,0

Automated extraction of tea: 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)	LOQ (µg/kg)	Rec. (%) (*n* = 3)
AM01	AcN	10	-	120	40	No	-	10	2.50	-	20	46
AM02	AcN	10	-	120	40	No	-	10	2.50	PSA	20	49
AM03	AcN	10	-	120	40	No	-	10	2.50	PSA, FA	20	54
AM04	AcOEt	10	-	120	40	No	-	10	2.50	-	20	27
AM05	AcOEt	10	-	120	40	No	-	10	2.50	PSA	20	29
AM06	AcOEt	10	-	120	40	No	-	10	2.50	PSA, FA	20	28
AM07	AcN	10	60	60	40	No	-	10	2.50	-	> 20	-
AM08	AcN	10	90	60	40	No	-	10	2.50	-	20	31
AM09	AcN	5	60	60	40	Yes	5	10	2.50	-	20	41
AM10	AcN	10	-	90	40	Yes	5	15	3.75	-	10	78
AM11	AcN	10	30	90	40	Yes	5	15	3.75	-	20	57
AM12	**AcN**	**10**	**-**	**150**	**40**	**Yes**	**5**	**15**	**3.75**	**-**	**7.5**	**101**

AcN *= acetonitrile;* ***AcOEt*** *= ethyl acetate;* ***FA*** *= formic acid;* ***PSA*** *= primary secondary amine*

Automated extraction of tea: 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)	LOQ (µg/kg)	Rec. (%) (*n* = 3)
AM01	AcN	10	-	120	40	No	-	10	2.50	-	20	46
AM02	AcN	10	-	120	40	No	-	10	2.50	PSA	20	49
AM03	AcN	10	-	120	40	No	-	10	2.50	PSA, FA	20	54
AM04	AcOEt	10	-	120	40	No	-	10	2.50	-	20	27
AM05	AcOEt	10	-	120	40	No	-	10	2.50	PSA	20	29
AM06	AcOEt	10	-	120	40	No	-	10	2.50	PSA, FA	20	28
AM07	AcN	10	60	60	40	No	-	10	2.50	-	> 20	-
AM08	AcN	10	90	60	40	No	-	10	2.50	-	20	31
AM09	AcN	5	60	60	40	Yes	5	10	2.50	-	20	41
AM10	AcN	10	-	90	40	Yes	5	15	3.75	-	10	78
AM11	AcN	10	30	90	40	Yes	5	15	3.75	-	20	57
AM12	**AcN**	**10**	**-**	**150**	**40**	**Yes**	**5**	**15**	**3.75**	**-**	**7.5**	**101**

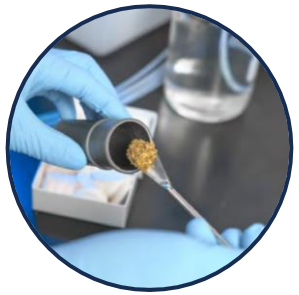
- AcN was the most efficient solvent
- Bubbling (agytation) was deemed counterproductive
- A rinse step significantly improved recovery values
- LOQ set at 0.0075 mg/kg (MRL = 0.02 mg/kg)

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

Automated extraction of tea: final method

EPRW 2020 · GRANADA

1

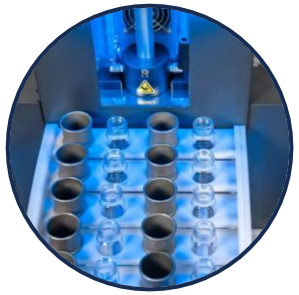


4 g tea

Place filters (Q-Disc) in tube

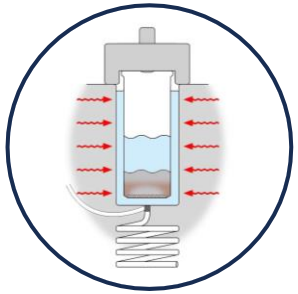
Cover sample with sand or Q-Screen

2



10 mL AcN addition

3

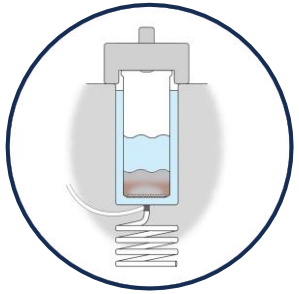


Hold 150 s at 40 °C

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

Evaporate 50 µL of the extract and dissolve in AcOEt

6

GC-MS/MS Analysis

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

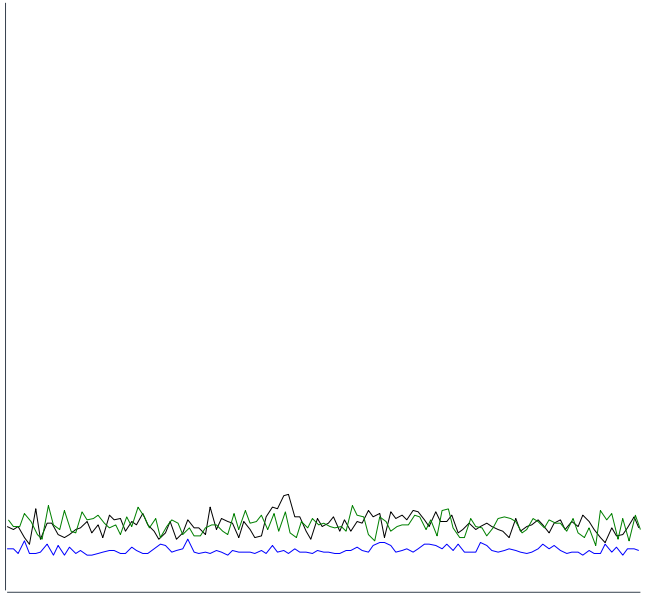
EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

UNIVERSIDAD DE ALMERÍA

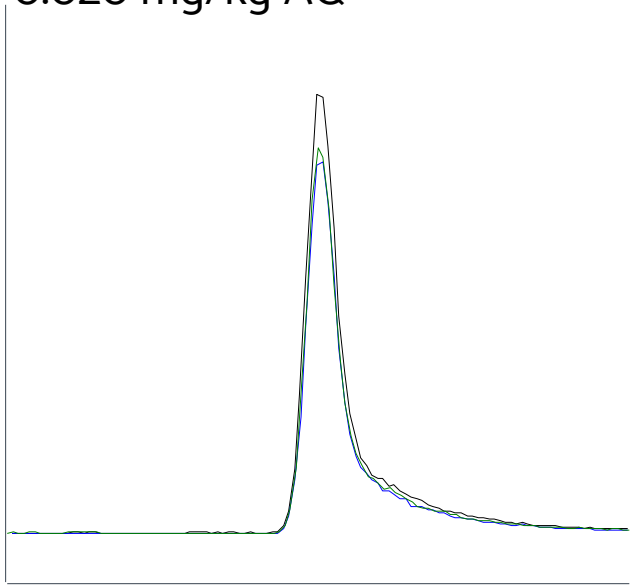
Automated extraction of tea: final method

- Extraction using pressurized liquids avoids the coextraction of matrix interferences

Tea blank

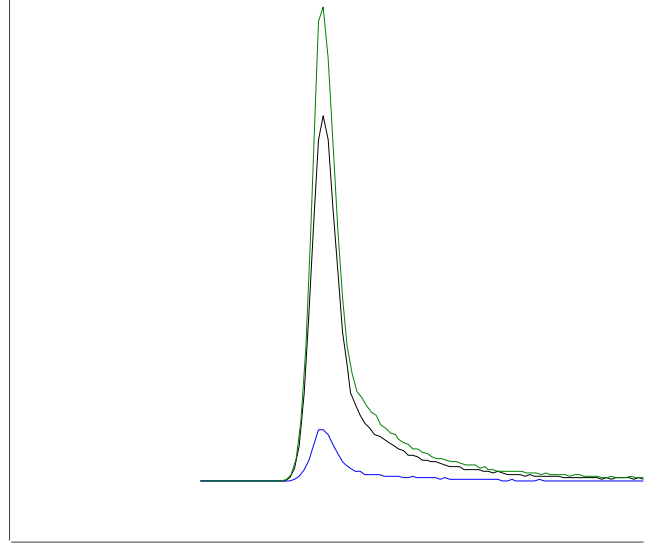


Tea blank spiked with 0.020 mg/kg AQ



208,0 -> 152,0 , 208,0 -> 180,0 , 180,0 -> 152,0

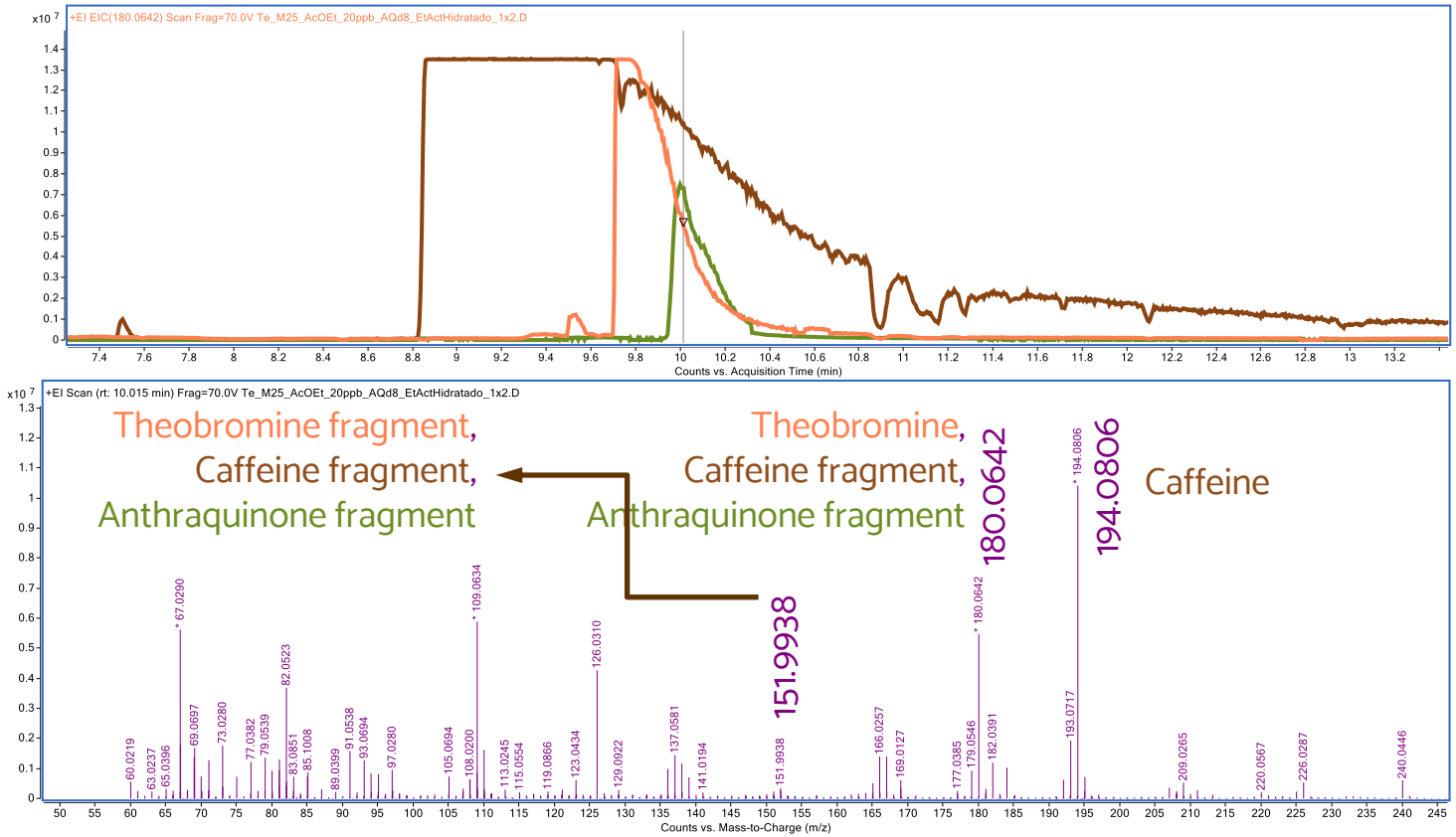
Tea blank spiked with 0.020 mg/kg AQ-D_8



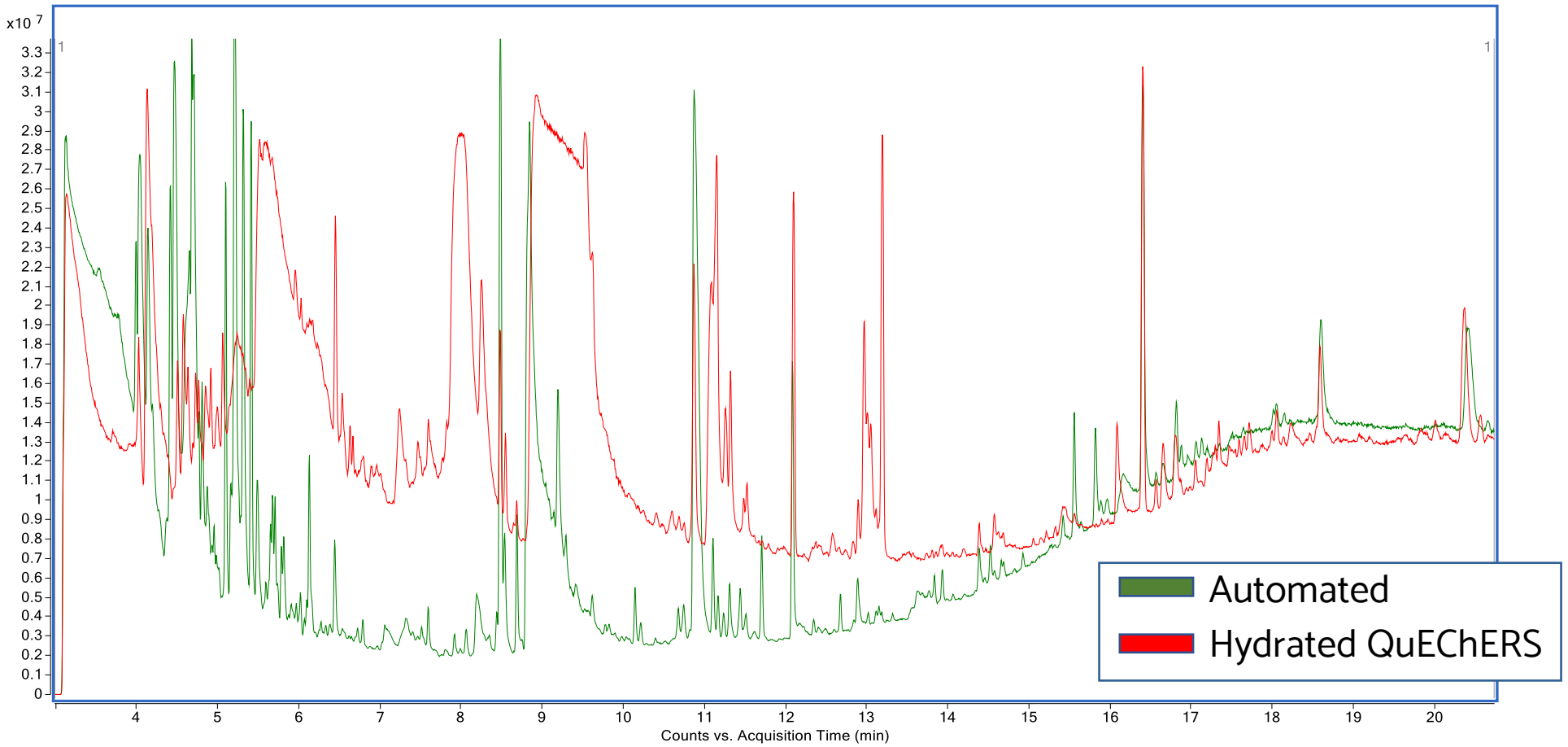
216,0 -> 160,0 , 216,0 -> 188,0 , 188,0 -> 160,0

Automated extraction of tea: final method

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

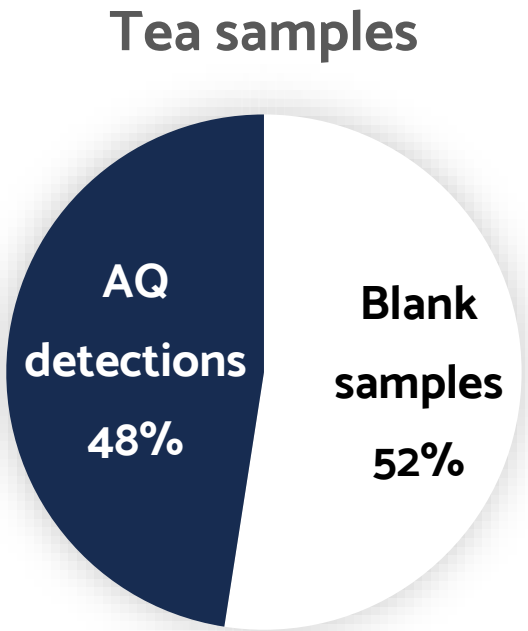


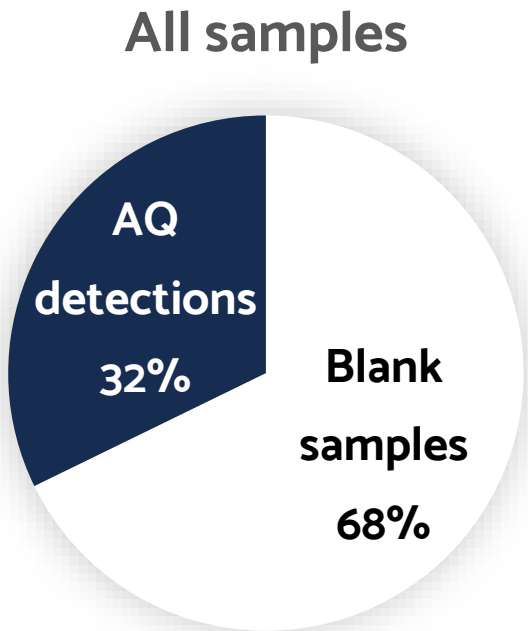
Total ion chromatogram (HRAMS): lower baseline achieved



Sampling study of tea and dry herbs

- In a sampling study of 90 real samples, AQ was detected in 32 % of all samples (48 % in tea) at levels below the current MRL (0.02 mg/kg)





Ring test successful participation

- The automated method was evaluated in a ring test of AQ in black tea, with a *z*-score of 0.1

Ring test P2001-RT

Polar pesticides and contaminants in black tea

January 2020

EURLFV European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables

Almeria, Spain

Laboratory code

Parameter	Result [mg/kg]	z-score	Comparability criterion passed	Result in % of the spiked level	Trueness criterion passed
Anthraquinone	0.0481	0.1	yes	107	yes

2 Automated extraction of cocoa and coffee

EPRW
2020 · GRANADA

Automated extraction of cocoa and coffee beans

EPRW
2020 · GRANADA

- The method was tested and successfully validated for cocoa and coffee beans, and included LC and GC amenable pesticide residues

Automated sample extraction
(*EDGE*)

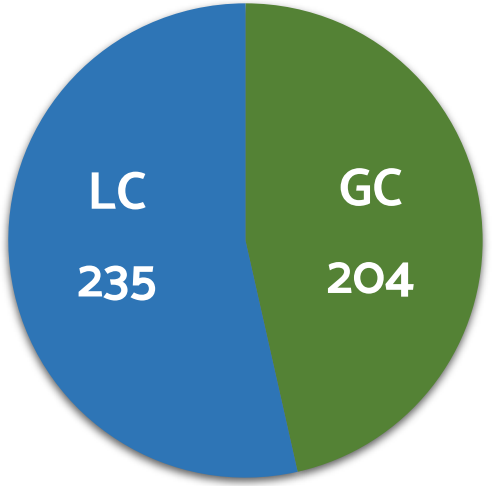
LC-MS/MS
(*SCIEX Exion LC & 6500+*)

GC-MS/MS
(*Agilent Intuvo & 7010B*)

Cocoa and coffee: pesticide residues evaluated

EPRW
2020 · GRANADA

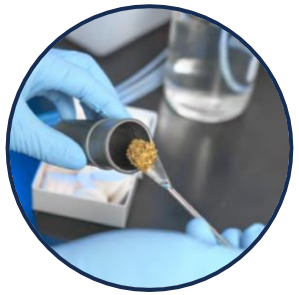
- **363** unique pesticide residues were evaluated by LC and GC
- In sum, **235** pesticide residues were evaluated by **LC-QqQ-MS/MS** and **204** by **GC-QqQ-MS/MS**
- For pesticides both LC and GC amenable, validation was performed with both techniques
- Evaluation performed at 0.010 and 0.050 mg/kg
  - Mean recovery ($n = 5$)
  - Within-laboratory reproducibility expressed as RSD_r
  - Matrix effect was also studied



Cocoa and coffee: automated extraction & GC analysis

EPRW
2020 · GRANADA

1

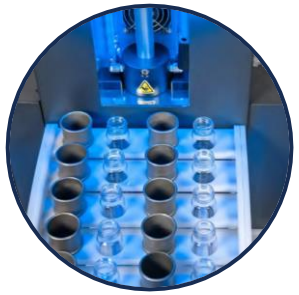


4 g coffee/cocoa

Place filters (Q-Disc) in tube

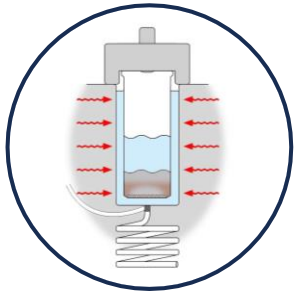
Cover sample with sand or Q-Screen

2



10 mL AcN addition

3

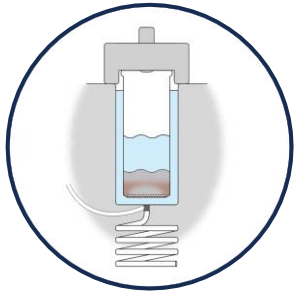


Hold 150 s at 40 °C

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

Evaporate 50 µL of the extract and dissolve in AcOEt

6

GC-MS/MS Analysis

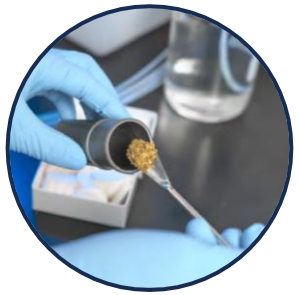
EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

UNIVERSIDAD DE ALMERÍA

Cocoa and coffee: automated extraction & LC analysis

EPRW
2020 · GRANADA

1

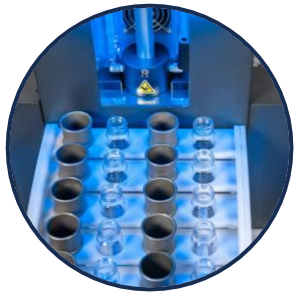


4 g coffee/cocoa

Place filters (Q-Disc) in tube

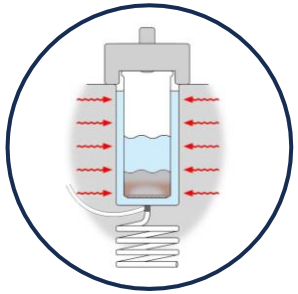
Cover sample with sand or Q-Screen

2



10 mL AcN addition

3

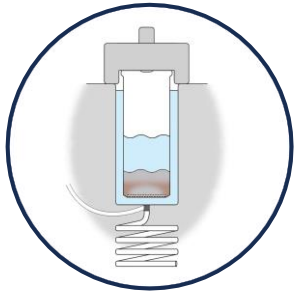


Hold 150 s at 40 °C

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

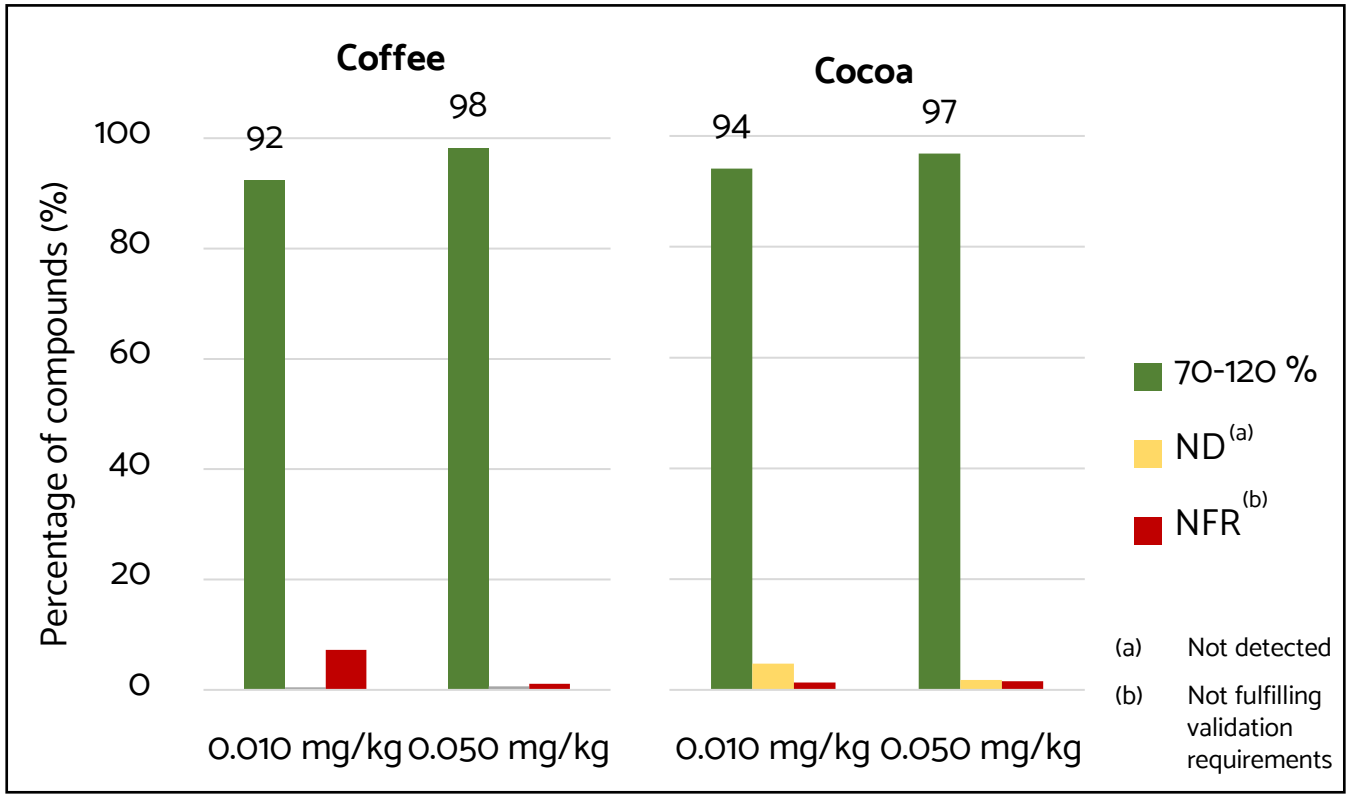
LC-MS/MS Analysis

EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

UNIVERSIDAD DE ALMERÍA

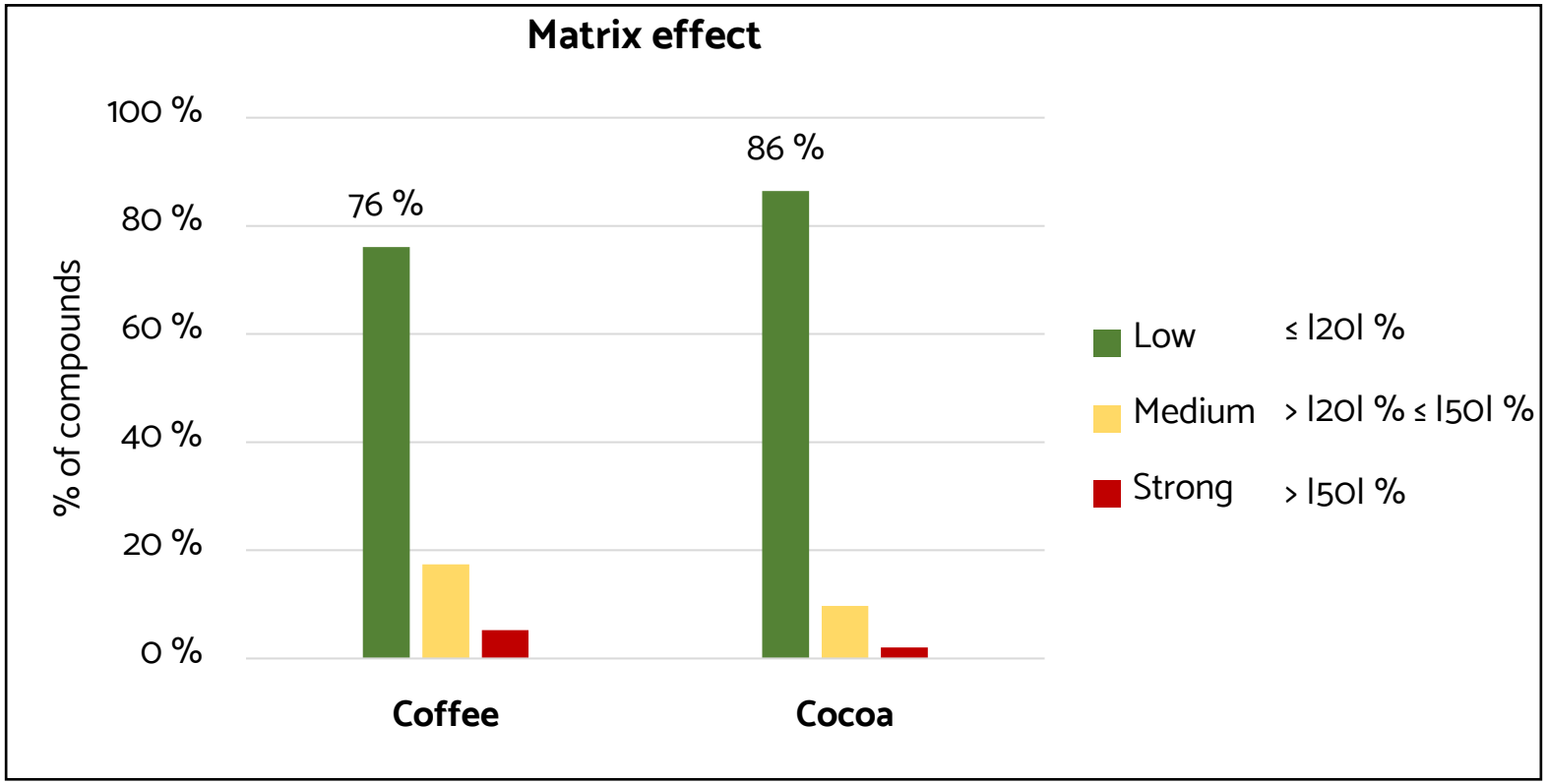
Cocoa and coffee: results for the automated method

- 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



Cocoa and coffee: manual extraction & GC analysis

EPRW 2020 · GRANADA

- Again, the developed automated extraction method was compared against a QuEChERS extraction of cocoa and coffee beans

5 g coffee/cocoa 1
+
5 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
5 mL extract
+ 750 mg $MgSO_4$ + 125 mg C_{18}
+ 125 mg PSA

→ Vortex 30 s
Centrifuge 5 min →

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

→ Evaporate 50 µL of the extract and dissolve in AcOEt

ANALYSIS 5

GC parameters
Agilent Intuvo 9000 system
2 planar columns
(15 m × 0.25 mm × 0.25 µm)
Splitless injection
Total runtime 12.4 min
Injection volume 1 µL

MS parameters
Agilent 7010B Triple Quad
Ionization mode: EI
Acquisition mode: dMRM

EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

UNIVERSIDAD DE ALMERÍA

Cocoa and coffee: manual extraction & LC analysis

- Again, the developed automated extraction method was compared against a QuEChERS extraction of cocoa and coffee beans

5 g coffee/cocoa 1
+
5 mL H_2O (*wait 30 min*)
+
10 mL AcN

→ Shake 7 min

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

→ Shake 7 min
Centrifuge 5 min

dSPE: 3
5 mL extract
+ 750 mg $MgSO_4$ + 125 mg C_{18}
+ 125 mg PSA

→ Vortex 30 s
Centrifuge 5 min

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

→ Dilute 1:4 with H_2O

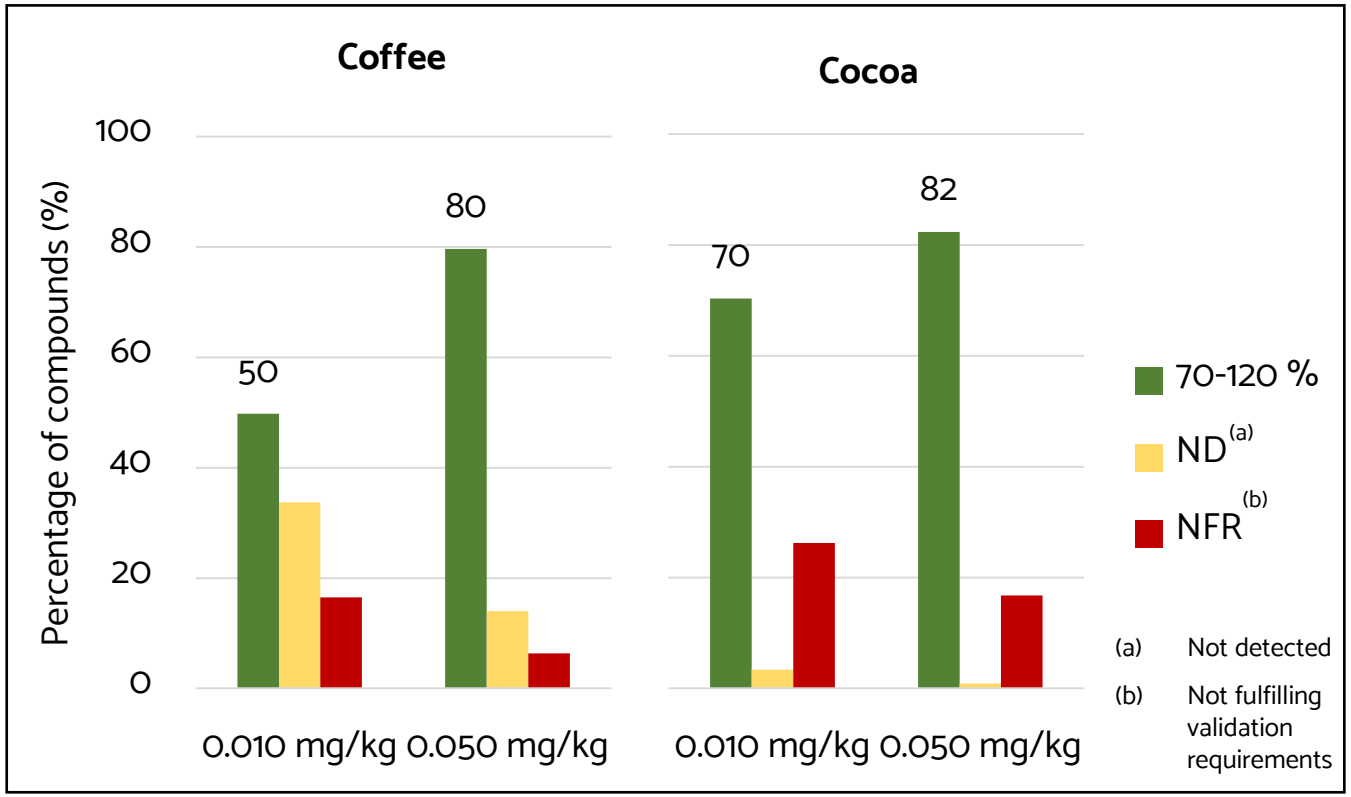
ANALYSIS 5

LC parameters
SCIEX Exion LC
Zorbax C8 (Agilent)
(2 mm × 100 mm × 1.8 µm)
Mobile phase: H_2O/MeOH
Flow rate: 0.3 mL/min
Injection volume 2.5 µL

MS parameters
SCIEX 6500+
Acquisition mode: dMRM
Ionization: polarity switching
IonSpray Voltage (+): 5500 V
IonSpray Voltage (-): -4500 V
Temperature: 300 °C

Cocoa and coffee: results for the hydrated QuEChERS method

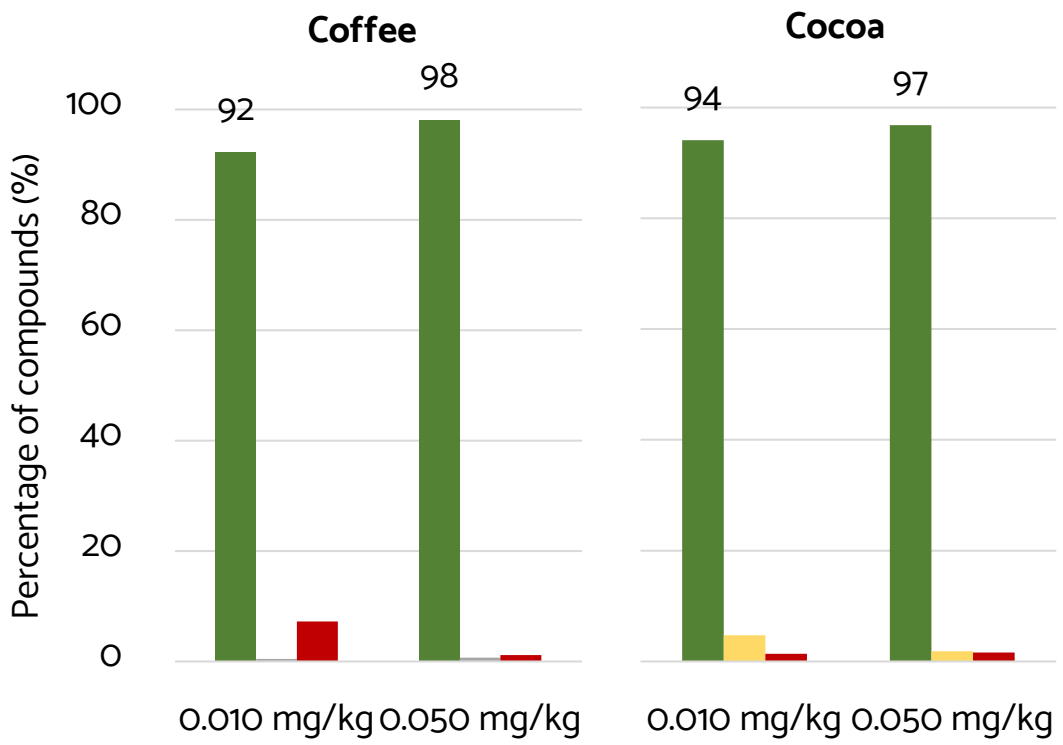
- Far fewer compounds could be successfully validated with this method. Worth noting the high number of non-detections in the case of coffee



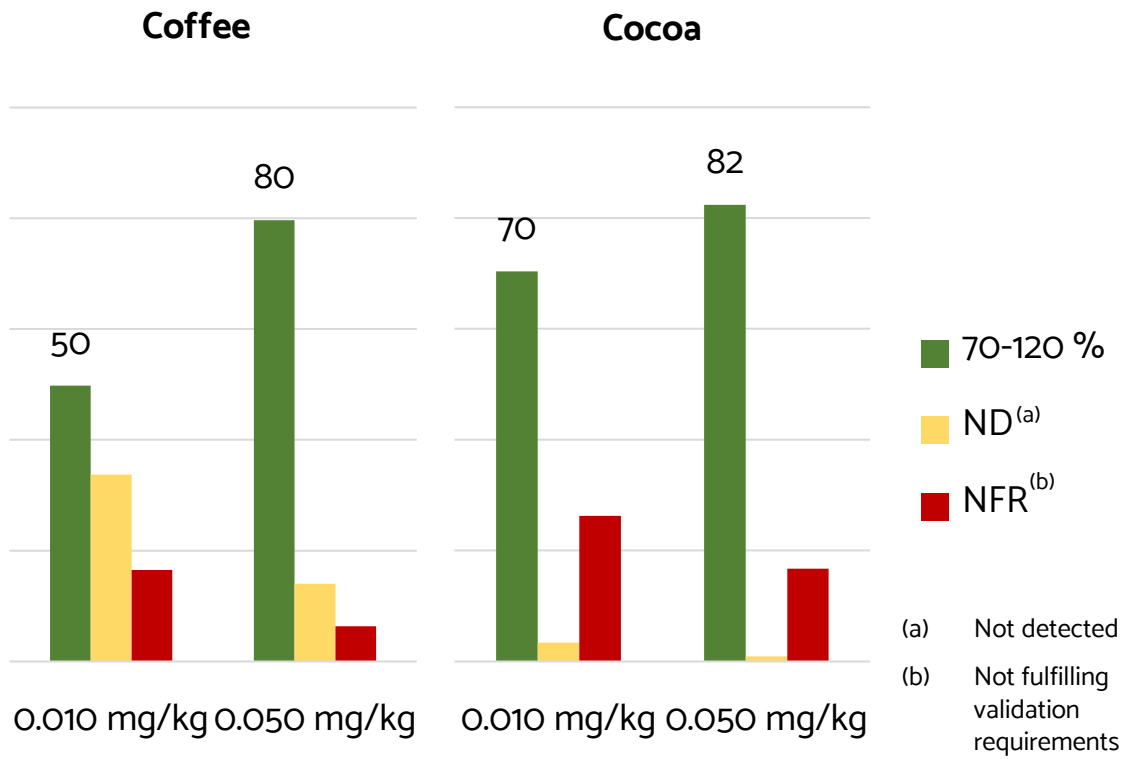
Cocoa and coffee: a comparison between extraction methods

EPRW 2020 · GRANADA

Automated extraction
(*Pressurized liquid extraction*)



Manual extraction
(QuEChERS with hydration)

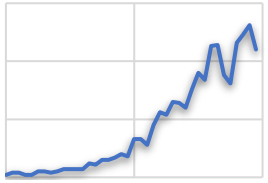


EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

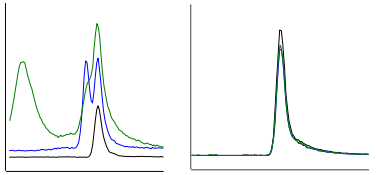
UNIVERSIDAD DE ALMERÍA

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 anthraquinone in tea and other dry herbs

- This technique also provides better results in the extraction of pesticide residues from cocoa and coffee beans

Future work

- Perform a sampling study of real cocoa and coffee samples

- Extend the method to other matrixes such as avocado or palm oil

- Extend the method to other pesticides, such as those QuPPE amenable

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

EPRW
2020 · GRANADA

Thank you

EURL
EUROPEAN UNION REFERENCE LABORATORY
FRUITS AND VEGETABLES