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

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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

Automated extraction of tea: the case of anthraquinone

• 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

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

1. 2 g tea + 4 mL H₂O (wait 30 min) + 10 mL AcN
   - Shake 7 min

2. 4 g MgSO₄, 1 g NaCl, 1 g Na₃Citrate·2 H₂O, 0.5 g Na₂HCitrate·1.5 H₂O
   - Shake 7 min
   - Centrifuge 5 min

3. dSPE:
   - 3 mL extract + 150 mg CaCl₂ + 150 mg PSA
   - Centrifuge 5 min

4. Transfer supernatant into 4 mL vial + add 30 μL formic acid (5 %)
   - Vortex 30 s
   - Centrifuge 5 min

5. ANALYSIS
   - 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

Automated extraction of tea: problems with manual extraction

- Tea hydration causes the coextraction of matrix components that hinder the analysis of AQ.
### Automated extraction of tea: method optimization

<table>
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<tr>
<th>Method (AMXX)</th>
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<th>T (°C)</th>
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<th>Rinse volume (mL)</th>
<th>Total solvent (mL)</th>
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<th>Clean-up (dSPE)</th>
<th>LOQ (µg/kg)</th>
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*AcN = acetonitrile; AcOEt = ethyl acetate; FA = formic acid; PSA = primary secondary amine*
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• 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)
Automated extraction of tea: final method

1. Place filters (Q-Disc) in tube
2. 10 mL AcN addition
3. Hold 150 s at 40 ºC
4. Dispense into collection tube
5. Rinse with 5 mL AcN
6. 7 min total run

Rinse with 5 mL AcN
Evaporate 50 µL of the extract and dissolve in AcOEt

Up to 70 samples/8 hr

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

Tea blank spiked with 0.020 mg/kg AQ-D₈

208.0 → 152.0, 208.0 → 180.0, 180.0 → 152.0

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
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)
The automated method was evaluated in a ring test of AQ in black tea, with a z-score of 0.1.
2 Automated extraction of cocoa and coffee
Automated extraction of cocoa and coffee beans

- The method was tested and successfully validated for cocoa and coffee beans, and included LC and GC amenable pesticide residues
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: automated extraction & GC analysis

1. 4 g coffee/cocoa
   - Place filters (Q-Disc) in tube
   - Cover sample with sand or Q-Screen

2. 10 mL AcN addition
   - Hold 150 s at 40 ºC
   - Dispense into collection tube

3. P ≈ 2 bar
   - Evaporate 50 µL of the extract and dissolve in AcOEt
   - GC-MS/MS Analysis

4. Rinse with 5 mL AcN
   - Dispense into collection tube

5. 7 min total run
   - Up to 70 samples/8 hr

6. EPRW 2020 · GRANADA
Cocoa and coffee: automated extraction & LC analysis

1. Place filters (Q-Disc) in tube
   - 4 g coffee/cocoa

2. Cover sample with sand or Q-Screen
   - 10 mL AcN addition

3. Hold 150 s at 40 °C
   - Dispense into collection tube
   - P ≈ 2 bar

4. Dispense into collection tube
   - Rinse with 5 mL AcN

5. 7 min total run
   - Up to 70 samples/8 hr

6. LC-MS/MS Analysis
   - Dilute 1:4 with H₂O
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

![Matrix effect diagram]

<table>
<thead>
<tr>
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<th>Coffee</th>
<th>Cocoa</th>
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<td>% of compounds</td>
<td>76 %</td>
<td>86 %</td>
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- Low: ≤ 120 %
- Medium: > 120 % ≤ 150 %
- Strong: > 150 %
Cocoa and coffee: manual extraction & GC analysis

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

1. 5 g coffee/cocoa
   + 5 mL H₂O (wait 30 min)
   + 10 mL AcN
   Shake 7 min

2. 4 g MgSO₄, 1 g NaCl, 1 g Na₃Citrate·2 H₂O, 0.5 g Na₂HCitrate·1.5 H₂O
   Shake 7 min
   Centrifuge 5 min

3. dSPE:
   5 mL extract
   + 750 mg MgSO₄ + 125 mg C₁₈
   + 125 mg PSA
   Centrifuge 5 min

4. Transfer supernatant into 4 mL vial + add 30 μL formic acid (5%)
   Evaporate 50 μL of the extract and dissolve in AcOEt

5. ANALYSIS
   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

Vortex 30 s
Centrifuge 5 min
Cocoa and coffee: manual extraction & LC analysis

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

1. 5 g coffee/cocoa + 5 mL H₂O (*wait 30 min*) + 10 mL AcN
   - Shake 7 min

2. 4 g MgSO₄, 1 g NaCl, 1 g Na₃Citrate-2 H₂O, 0.5 g Na₂HCitrate-1.5 H₂O
   - Shake 7 min
   - Centrifuge 5 min

3. dSPE:
   - 5 mL extract + 750 mg MgSO₄ + 125 mg C₁₈ + 125 mg PSA
   - Centrifuge 5 min

4. Transfer supernatant into 4 mL vial + add 30 μL formic acid (5 %)
   - Dilute 1:4 with H₂O

5. ANALYSIS MS parameters
   - SCIEX 6500+
   - Acquisition mode: dMRM
   - IonSpray Voltage (+): 5500 V
   - IonSpray Voltage (-): -4500 V
   - Temperature: 300 ºC

   LC parameters
   - SCIEX Exion LC
   - Zorbax C8 (Agilent)
   - (2 mm × 100 mm × 1.8 μm)
   - Mobile phase: H₂O/MeOH
   - Flow rate: 0.3 mL/min
   - Injection volume: 2.5 μL
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

Automated extraction
(Pressurized liquid extraction)

Manual extraction
(QuEChERS with hydration)

[Graphs showing percentage of compounds for coffee and cocoa under automated and manual extraction methods at different concentrations (0.010 mg/kg and 0.050 mg/kg).]

- Automated extraction:
  - Coffee: 92%, 98%
  - Cocoa: 94%, 97%

- Manual extraction:
  - Coffee: 50%, 80%
  - Cocoa: 70%, 82%

Legend:
- 70-120 %
- ND (a)
- NFR (b)

(a) Not detected
(b) Not fulfilling validation requirements
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
Thank you