SPE and QuEChERS – Method Development

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Christophe Deckers
Today’s Agenda

1. QuEChERS Workflow overview and original methods
2. Method development for alternative matrices
3. SPE for polar compounds
4. SPE for non-polar compounds
5. SPE for ionic compounds
6. Questions
### Filtration and Other Sample Preparation Techniques

<table>
<thead>
<tr>
<th>Sample Prep Technique</th>
<th>Dilute &amp; Shoot</th>
<th>Filtration</th>
<th>Liquid/Liquid Extractions</th>
<th>Supported Liquid Extractions (SLE)</th>
<th>Dried Matrix Spotting</th>
<th>Precipitation</th>
<th>QuEChERS</th>
<th>Lipid Removal 'Hybrid' Filtration</th>
<th>Solid Phase Extraction</th>
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<tbody>
<tr>
<td>Lipids</td>
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<tr>
<td>Suggested Agilent Product</td>
<td>Agilent Autosampler Vials</td>
<td>Captiva Syringe Filters</td>
<td>Chem Elut</td>
<td>Bond Elut DMS</td>
<td>Captiva ND</td>
<td>Bond Elut QuEChERS</td>
<td>Captiva ND LIPIDS</td>
<td>Bond Elut Silica and Polymeric SPE</td>
<td></td>
</tr>
</tbody>
</table>

**Agilent Captiva Filtration Products are recommended for use with any LC or LC-MS method**
What is QuEChERS (pronounced “Catchers”)

Quick, Easy, Cheap, Effective, Robust and Safe

- Developed jointly by USDA and EU Food Regulatory Agencies as a sample preparation method for multi-residue analyses
- Simplified extraction and cleanup approaches that reduce use of expensive and/or dangerous solvents
- Originally for preparing fruits and vegetables for pesticide analysis
- Rapidly being extended to other matrices and compound classes
## Time = Money?

<table>
<thead>
<tr>
<th></th>
<th>Luke method, traditional SPE, or GPC</th>
<th>QuEChERS</th>
<th>QuEChERS Benefits!</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated Time to process 6 samples (min)</td>
<td>120</td>
<td>20</td>
<td>6 x faster</td>
</tr>
<tr>
<td>Solvent Used (mL) per sample</td>
<td>90 mL</td>
<td>10-15mL</td>
<td>9 x less solvent</td>
</tr>
<tr>
<td>Chlorinated Waste (mL)</td>
<td>30 mL</td>
<td>none</td>
<td>safer, greener, less costly</td>
</tr>
<tr>
<td>Glassware/ specialized equipment</td>
<td>Clean Separatory funnels, water bath, 200mL containers, evaporator, etc.</td>
<td>None</td>
<td>No additional supplies needed</td>
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</table>

Significant time savings because lengthy liquid extraction procedures are eliminated!
THE ORIGINAL QUECHERS METHOD

Pesticide Residue in Fruit and Vegetables
QuEChERS Extraction Flow Chart

AOAC

15 gm homogenized sample + IS

Add 15 mL ACN (1% AA), vortex

Add AOAC Extraction Salts, shake

Centrifuge

Transfer 1 or 8 mL to AOAC d-SPE tube, vortex, centrifuge

Transfer to Analysis vial

EN

10 gm homogenized sample + IS

Add 10 mL ACN, vortex

Add EN Extraction Salts, shake

Centrifuge

Transfer 1 or 6 mL to EN d-SPE tube, vortex, centrifuge

Transfer to Analysis vial

Analyze by GC/MS or LC/MS/MS*

* Requires a dilution prior to analysis
Sample Homogenization – Pre-Preparation

- Frozen sample
- Initial milling without dry ice
- Add dry ice, continue milling
- Stir while milling
- Gradually increase milling speed
QuEChERS – Easy as 1-2-3

**Step 1: Extract**
- Weigh sample
- Add solvent
- Shake
- Add salts
- Add internal standard
- Shake and centrifuge
- Transfer extract (top) for cleanup

**Step 2: Clean**
- Shake and centrifuge

**Step 3: Analyze**
- Transfer (dilute or concentrate) to vials

*LC-GC, 2008, vol. 11 issue 1*
Agilent Tools for Pesticide Residue Analysis
….but I don’t LIKE vegetables!!
ALTERNATIVE MATRICES

Method Development
“Trial and Error” vs. “Educated Guess”

Trial and Error for Extraction Step:
• Only three existing methodologies
• Unpredictability of results
• Eliminates need for bulk salts

Educated Guess for Clean Up Step
• Predictability of results
• Better understanding = less time and $ developing methods!
Optimization Considerations for Juice Concentrates - A Case Study

- Extraction and Dispersive SPE
- Sample amount
- pH variation (Lemon juice is highly acidic)
- AP (analyte protectant)

*Juice concentrates are a distinctively challenging matrix due to pH and consistency*
Optimization of QuEChERS Procedure: Extraction Salt Selection

• Three variations of the QuEChERS extraction salts were investigated
  - Original, Non-buffered: 4 g MgSO$_4$, 1 g NaCl
  - AOAC: 6 g MgSO$_4$, 1.5 g NaAc
  - EN: 4 g MgSO$_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate

**TIP!**
Use one dSPE mixture and keep this part the same for the extraction salt optimization
QuEChERS Extraction Optimization Summary

Use one dSPE type with three salt types to identify the best combination for the application

Method Development Process

- Select Representative Sample
- Homogenize
- Weigh
- Mix with salts, solvent
- Centrifuge
- Transfer aliquots of supernatant to one of the dSPE tubes
- Mix
- Centrifuge
- Transfer supernatant to vials
- Analyze
- Identify best extraction salts

Method Development Products – Fruits & Vegetables

- dSPE Mix 1
- EN Extraction Salts 5982-5650CH
- Original 5982-5550CH
- AOAC 2007.01 5982-5755CH
dSPE Selection: Educated Guess

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO4</td>
<td>Present in all QuEChERS kits, removes residual water</td>
</tr>
<tr>
<td>PSA</td>
<td>“Primary/Secondary Amine” scavenges organic acids and sugars, typical matrix component in fruits and vegetables</td>
</tr>
<tr>
<td>C18</td>
<td>scavenges residual proteins and lipids, amount in kits appropriate for f&amp;v, may need adjustment</td>
</tr>
<tr>
<td>GCB</td>
<td>“graphitized carbon black”, removes pigments (notably chlorophyll and carotenoids)</td>
</tr>
</tbody>
</table>
dSPE Selection for Juice Concentrate

- EN extraction salt = EN dSPE kit because ratios matter
- No lipids and proteins = no need for C18
- No considerable pigmentation = no need for GCB
- Significant organic acids and sugars

EN Fruits and Vegetables
5982-5021CH
QuEChERS Optimization
- Sample Amount Variation

• Overall sample volume (sample plus water) MUST be 10ml or 15ml (EN vs. AOAC)
• Sample amount ↑
  - Extracted compound amount ↑ → helps reaching low detection limits
  - GC-MS/MS contamination ↑ → not desired
• Lemon juice concentrate was spiked at 100 ppb and 3, 5, 7 g of sample loading amounts were tested
• For some compounds (e.g. Dichlofluanid, Tolylfluanid, Captan, Folpet) drastically better response from 2 – 6 times higher when 5 g of sample were used compared to 3 g of sample

→ Optimized method with 4g of sample
QuEChERS Optimization - pH Variation

- pH value is below 2 in the lemon juice concentrate and some compounds are not recovered from the extraction step.
- pH variation experiment was done to find the right pH range for extraction step.
- 0, 0.6, 1, 2 mL of 5 N NaOH was used for pH variation in the extraction step.
- With pre-spiked lemon juice concentrate (100 ppb), different pH values were tested for recovery and peak shape.
QuEChERS Optimization - pH Variation

**Omethoate**
- 0 mL 5 N NaOH
- 0.6 mL 5 N NaOH
- 1 mL 5 N NaOH
- 2 mL 5 N NaOH

**Captan**
- 0 mL 5 N NaOH
- 0.6 mL 5 N NaOH
- 1 mL 5 N NaOH
- 2 mL 5 N NaOH

**Atrazine**
- 0 mL 5 N NaOH
- 0.6 mL 5 N NaOH
- 1 mL 5 N NaOH
- 2 mL 5 N NaOH

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QuEChERS Optimization - pH Variation

**Buprofezin**
- 0 mL 5 N NaOH
- 0.6 mL 5 N NaOH
- 1 mL 5 N NaOH
- 2 mL 5 N NaOH

**Piperonyl Butoxide**
- 0 mL 5 N NaOH
- 0.6 mL 5 N NaOH
- 1 mL 5 N NaOH
- 2 mL 5 N NaOH

**Fenarimol**
- 0 mL 5 N NaOH
- 0.6 mL 5 N NaOH
- 1 mL 5 N NaOH
- 2 mL 5 N NaOH
QuEChERS Optimization - pH Variation

- Problematic compounds showed improved recovery with 5 N NaOH.
- Amount of 5 N NaOH affects recovery. When tested with 0, 0.6, 1, and 2 mL of 5 N NaOH, overall 2 mL 5 N NaOH addition showed the best performance when 4 g of sample was used. Only Captan showed better recovery when 0.6 mL of 5 N NaOH was used.
- Some compounds almost completely disappeared when no 5 N NaOH was added such as Omethoate, Atrazine, Buprofezin, Bupirimate, Piperonyl Butoxide, Fenarimol.

→ Use 2 mL of 5 N NaOH in the extraction step to raise the pH to ~5.
QuEChERS Optimization – AP (Analyte Protectant)


- Many compounds are available and suitable for AP and from practical point of view a mixture of D-sorbitol and L-gulonolactone is the best

- Add 50 mg of D-sorbitol and 100 mg of L-gulonolactone to 5 mL of ACN to make 10 mg/mL and 20 mg/mL concentration in the mix, respectively
QuEChERS Optimization – AP (Analyte Protectant)

Diazinon

Hexachlorobenzene

Ethion

APs are a must in multi-residue pesticide analysis
QuEChERS EN Method – Extraction Protocol Optimized for Juice Concentrates

• Add 4 g of lemon juice concentrate to EN 50 mL extraction tubes

• Spike 80 µL of standard mix in ACN + 1% acetic acid, shake for 10 min

• Add 6 mL of water to EN extraction tubes (to make the total sample loading 10 g)

• Add 2 mL of 5 N NaOH solution for pH adjustment

• Add 10 mL of ACN to EN extraction tubes and vortex briefly, add Bond Elut EN salt packet and ceramic homogenizers

• Shake for 1 minute, then centrifuge at 4,000 RPM for 2 min
General considerations for alternative matrices or target compounds

- Dried material (e.g. teas, herbs): use less sample, adjust with water, pre-soaking can help recoveries
- If target compounds are acidic, consider PSA-free kit
- Matrices from animal sources tend to be protein and lipid rich, dSPE should contain C18
- Acidifying ACN can help reduce secondary interactions (e.g. protein binding)
- dSPE amount in tubes may need to be adjusted/supplemented (or substitute SPE)
Agilent SPE for Ultimate Cleanliness

Method Development
The Four Steps of SPE – Selective Elution

Green = Blue and Yellow

Blue is more non polar than yellow

Blue is retained
Is Your Target Compound….

<table>
<thead>
<tr>
<th></th>
<th>Log P &lt; 1.5</th>
<th>Polar (lp), Ion Exchange (?) (aq, lp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Polar</strong></td>
<td>Log P &gt; 1.5 and &lt; 4</td>
<td>Non-Polar (aq), Ion Exchange (?) (aq, lp), Polar (Ip)</td>
</tr>
<tr>
<td><strong>Moderate Polarity</strong></td>
<td>Log P &gt; 4</td>
<td>Non-polar (aq), might need lipid clean up, polar unless hydrocarbon</td>
</tr>
<tr>
<td><strong>Non-Polar</strong></td>
<td>pKa &lt;2 or &gt;11</td>
<td>Weak anion or cation exchange or mixed-mode</td>
</tr>
<tr>
<td><strong>Strongly acidic or basic</strong></td>
<td>pKa &gt;2 and &lt;11</td>
<td>Strong anion or cation exchange or mixed-mode</td>
</tr>
</tbody>
</table>
Is Your Matrix

- Mostly aqueous (e.g. fruit juice, energy drinks, brewed teas)
- Mostly lipids or organics (e.g. olive oil, lotions, non-polar extracts)
- Polar extracts (MeOH or ACN): dry down or dilute
NON-POLAR EXTRACTIONS

Method Development
Interactions with Non-Polar Sorbents
Silica VS. Polymer

- “True” polar/ion exchange possible
- Wide range of chemistries
- Wide range of established methods
- Can be more selective

Polymer

- Inherent hydrophobicity (conditioning)
- Higher capacity (sorbent mass/flow)
- Polarity gradient in Plexa
Method Development Considerations

Solubility characteristics of target compound?

- Water
- Methanol
- IPA
- Acetonitrile
- Acetone

Polar

- Ethyl Acetate
- Ethyl Ether
- THF
- Dichloromethane
- Chloroform
- Toluene
- iso octane
- hexane

Non polar

Water miscible

Water immiscible
Method Development Considerations

- Select suitable solvents (water miscible only)
- Prepare 0%-100% concentrations
- Plot recoveries

Elution Profile

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Method Development Consideration

- Highest % organic with low recoveries for wash A
- Lowest % organic with high recoveries for elution B
- Try acid/base modifiers and MeOH/ACN mix
Low recovery even at 100% organic?

- Use stronger organic solvent, **dry cartridge** before elution step
- But stronger solvents often = more non-polar contaminants
- Make sure the isolate is soluble and does not degrade under the extraction conditions.
- Reduce secondary interactions on silica-based SPE with buffers addition at different pHs in elution solvent. Addition of 0.5% HCl can help with elution of amine groups.
- Consider lower hydrophobicity sorbent (e.g. CH, C2)
POLAR EXTRACTIONS

Method Development
Polar (dipole or H-bonding) Interactions

Silica base

- Packing is polar
- Mobile phase is non-polar (e.g. hexane, methylene chloride, ethyl acetate)
- lower polarity/higher organic for retention
- higher polarity/lower organic for elution
Method Development Consideration

- The goal is to clean up lipids and oils
- Select most non-polar solvent compatible with analyte and matrix, hexane is ideal
- Load extract or hexane/matrix mixture under low vacuum (sample must be water free and SPE cartridges must be well stored to avoid moisture)
- Rinse with 100% loading solvent for 2x column volumes
- Elute with loading solvent + polar modifier such as IPA (about 5-10%) at 2-4 ml/min. Make sure that your analyte is soluble in elution solvent.
ION EXCHANGE EXTRACTIONS

Method Development
**Ion Exchange Nomenclature**

**STRONG**: Ionic group is always charged (+ or -)

**WEAK**: Ionic group is variably charged (+ or -)

**CATIONS**: (+) Found in basic compounds

**ANIONS**: (-) Found in acidic compounds

Extract weak ions with strong exchangers and strong ions with weak exchangers!
Interactions on Ion Exchange Sorbents

Electrostatic attraction
Method Development Considerations

What is the pKa of your compound?

\[ \text{pK}_a = -\log K_a \]

and

\[ K_a = [A^-][H^+]/[HA] \]

• If pH=pKa, 50% of the compound is ionized and 50% is neutral
• To ensure full charge or full neutralization, employ the rule of 2
Interactions on Ion Exchange Sorbents

If the pKa=9 and the pH=9
Interactions on Ion Exchange Sorbents

If the pKa=9
and the pH=7
Interactions on Ion Exchange Sorbents

If the pKa=9 and the pH=11
Important Consideration for Ion Exchange

• Reduce ionic strength of “salty” matrices by dilution
• Consider competitive binding when choosing bed mass
• Remember that **ALL** polymeric exchangers are mixed-mode, elute in organic solvent
• Some organic should be present even with silica based ion exchangers because of carbon linkers
• Reduce flow rate at sample application because ion exchange is a relatively slow interaction
In conclusion

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3. SPE for polar compounds
4. SPE for non-polar compounds
5. SPE for ionic compounds
6. Questions
Technical Support

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1-800-227-9770, 3,3,3

www.agilent.com/chem/subscribe
Questions