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>Interference Removed</th>
<th>More Specific</th>
<th>Instrument Separation and Detection Specificity</th>
<th>Less Specific</th>
<th>Sample Preparation Specificity</th>
<th>More Specific</th>
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<tbody>
<tr>
<td></td>
<td>Dilute &amp; Shoot</td>
<td>Filtration</td>
<td>Liquid/Liquid Extractions</td>
<td>Supported Liquid Extractions (SLE)</td>
<td>Dried Matrix Spotting</td>
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<tr>
<td>Lipids</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Some</td>
<td>No</td>
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<tr>
<td>Oligomeric Surfactants</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Particulates</td>
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<td>No</td>
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<tr>
<td>Pigments</td>
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<td>Polar Organic Acids</td>
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<td>Proteins</td>
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<td>Yes</td>
<td>Yes</td>
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<td>Salts</td>
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<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</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>
</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>
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<tr>
<td>Chlorinated Waste (mL)</td>
<td>30 mL</td>
<td>none</td>
<td>safer, greener, less costly</td>
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<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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</tbody>
</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

1. Weigh sample
2. Add solvent
3. Shake
4. Add salts
5. Add internal standard
6. Shake and centrifuge
7. Transfer extract (top) for cleanup

Step 2 CLEAN
Choose a dispersive SPE Kit specific to your matrix.

8. Shake and centrifuge
9. Transfer (dilute or concentrate) to vials

Step 3 ANALYZE
Using 6400 Series Triple Quad LC/MS and 7000 Series Triple Quad GC/MS

LC-GC, 2008, vol. 11 issue 1
Agilent Tools for Pesticide Residue Analysis

Comminuted Sample: 10 g or 15 g

- Add Acetonitrile

SELECT EXTRACTION KIT

- Original Method
  - 10 g samples
- Original Method
  - 15 g samples
- Buffered AOAC 2007.1 Method
  - 15 g samples
- Buffered EN 15662 Method
  - 10 g samples

Check pH and adjust to 5.5 - 5.5
Add internal standard
Shake and centrifuge

Aliquot: 1 mL, 6 mL or 8 mL

SELECT DISPERSIVE SPE KIT

- General Fruits & Vegetables
  - 2 mL and 15 mL kits
- Fatty/Waxy Fruits & Vegetables
  - 2 mL and 15 mL kits
- Pigmented Fruits & Vegetables
  - 2 mL and 15 mL kits
- High Pigment Fruits & Vegetables
  - 2 mL and 15 mL kits
- General Fruits & Vegetables
  - 2 mL and 15 mL kits
- Fatty/Waxy Fruits & Vegetables
  - 2 mL and 15 mL kits
- Pigmented Fruits & Vegetables
  - 2 mL and 15 mL kits
- Fruits & Vegetables with Fats, Pigments
  - 2 mL and 15 mL kits

AOAC METHOD
EN METHOD

Analysis
Shake and centrifuge
....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 MgSO₄, 1 g NaCl
  - AOAC: 6 g MgSO₄, 1.5 g NaAc
  - EN: 4 g MgSO₄, 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

MgSO4  -  Present in all QuEChERS kits, removes residual water

PSA    -  “Primary/Secondary Amine” scavenges organic acids and sugars, typical matrix component in fruits and vegetables

C18    -  scavenges residual proteins and lipids, amount in kits appropriate for f&v, may need adjustment

GCB    -  “graphitized carbon black”, removes pigments (notably chlorophyll and carotenoids)
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, Tolyfluanid, 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

Atrazine

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

- 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
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 elutions 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

Silica base

H\(_3\)N\(+\)\(\overset{-}{\mathrm{SO}}\)\(_3\)\(-\)\(\overset{+}{\mathrm{NH}}\)_3\(+\)

\(\overset{-}{\mathrm{CO}}\)\(_2\)\(-\)\(\overset{+}{\mathrm{N}}\)\(\overset{-}{\mathrm{O}}\)\(\overset{-}{\mathrm{CON}}\)(CH\(_3\))\(_2\)

\(\overset{-}{\mathrm{CO}}\)\(_2\)\(-\)\(\overset{+}{\mathrm{N}}\)\(\overset{-}{\mathrm{O}}\)\(\overset{-}{\mathrm{CON}}\)(CH\(_3\))\(_2\)

Electrostatic attraction
Method Development Considerations

What is the pKa of your compound?

\[ pK_a = -\log K_a \]

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

NH₃⁺ ↔ SO₃⁻

50%

50%

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Interactions on Ion Exchange Sorbents

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

If the $\text{pKa}=9$ and the $\text{pH}=11$
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