Seeing the Trees through the Forest

Analytical Challenges and Strategies for Residue Analysis in Cannabis

Rick Jordan
Pacific Agricultural Lab
Sherwood, OR
Pacific Agricultural Laboratory

- Established in 1995
- Pesticide Residue Laboratory
- Located in Sherwood, OR 18 miles SW of Portland
- Occupies 16,000 sf (10,000 sf laboratory, 6,000 office/storage)
- 14 employees
- Process 6500 samples/year, 13,000 analyses/year
Challenges of Residue Analysis in Cannabis

- Co-extracted Materials
  - 60+ Cannabinoids
  - 100+ Terpenes
  - Chlorophyll

Percent level interferences
Parts per billion target compounds

Which Residues?

Which Residues?

State “Regulatory” List

Multi-Residue Approach

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Naples FL, July 23-26, 2017
Why adopt multi-residue approach?

It is illegal to use ANY pesticide on cannabis not found on ODA’s cannabis and pesticide guidelist, and the pesticide product MUST be used according to the label directions.

Despite cannabis producers receiving test results below OHA pesticide action levels for cannabis (set in OHA rule), producers may still be in violation of the Oregon Pesticide Control Act if any levels of illegal pesticides are detected.
METHOD DEVELOPMENT

- Concise, efficient extraction
  - polymeric SPE pass through extraction/cleanup

- Dispersive cleanups tailored for specific analytical techniques
  - Individual d-SPE cleanups for GC-MS/MS and LC-MS/MS

- Optimize instrumentation to allow for large dilution factors
  - Control matrix effects using matrix matching

- Compound target list which cover state “regulatory” lists and additional prohibited substances
## State “Regulatory” List


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Comprehensive Screening

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Expanded Method Development for the Determination of Pesticides in Dried Hops by Liquid Chromatography with Tandem Mass Spectrometry

Matt J. Hengel, Department of Environmental Toxicology, University of California, Davis, CA

JASBC EXTRACTION

- 1.0 gram sample + 15mL Acetonitrile in a 50mL PP tube
- Add ceramic homogenizer and mechanically shake for 2 minutes
- Condition SPE column (Strata™-X 33µm, 500mg/12mL Gigatube) with 5mL acetonitrile
- Decant acetonitrile extract into SPE, collecting eluent in a second 50mL PP tube
- Rinse extraction tube with two 5ml portions of acetonitrile, transferring all solids to SPE
- Bring to 25mL final volume with acetonitrile (gravimetrically)
JASBC  
40 mg/mL  

QuEChERS  
40 mg/mL
INSTRUMENTATION AND ANALYSIS
RESIDUES BY GC-MS/MS

Agilent 7890 / 7010 GC-MS/MS

MMI
- Pulsed splitless injection (35psi)
- Cool inlet injection
  - 180°C → 280°C @ 400°C/min
  - 4mm single gooseneck liner with fused silica wool

Column Backflush
- Mid-run, when last compound passes Ultimate Union
- Postrun, 2.4 min @ 4.0 mL/min

Dissimilar Phase columns
- HP-35MS / HP-5MS
- 15m x 0.25mm x 0.25µm
RESIDUES BY GC-MS/MS

Calibration

• Matrix-matched calibration
  • Linear fit (min. 5 point) and quadratic fit (min. 6 point)

• Calibration range 0.2 ng/mL → 20 ng/mL

• 1/X weighting, exclude origin

• Correlation coefficient >0.990
Bifenthrin
$r^2 = 0.9979$

Ethoxyquin
$r^2 = 0.9996$

Dichlorvos
$r^2 = 0.9997$

Myclobutanil
$r^2 = 0.9984$
RESIDUES BY GC-MS/MS

Dispersive cleanup and dilution

100µL extract + 900µL hexane:acetone (1:1) into 2mL d-SPE

Agilent Universal d-SPE (5982-0028)
- 50 mg PSA
- 50 mg C18EC
- 7.5 mg graphitized carbon
- 150 mg MgSO₄

Vortex 30 sec
Centrifuge 2 min

300µL extract
300µL hexane:acetone 1:1
RESIDUES BY GC-MS/MS

Dispersive cleanup and dilution

100µL extract + 900µL hexane:acetone (1:1) into 2mL d-SPE

Vortex 30 sec
Centrifuge 2 min

300µL extract
300µL hexane:acetone 1:1

Final dilution factor of 20x for analysis
RESIDUES BY GC-MS/MS
GC-MS/MS RECOVERIES

0.2 mg/kg
n=5

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<table>
<thead>
<tr>
<th>Compound</th>
<th>% REC</th>
<th>% RSD</th>
<th>Compound</th>
<th>% REC</th>
<th>% RSD</th>
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<tr>
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<td>Fludioxonil</td>
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Bifenthrin, 0.2 ng/mL
Dichlorvos, 0.2 ng/mL
Ethoxyquin, 0.2 ng/mL
Myclobutanil, 0.2 ng/mL
RESIDUES BY LC-MS/MS

Agilent 1260 Infinity II Multisampler / 6470 LC-MS/MS

Poroshell 120 Phenyl-Hexyl 2.7 µm, 2.1mm x 100mm

Mobile Phase A
• 5mM ammonium formate with 0.1% formic acid in 95:5 water:methanol

Mobile Phase B
• 5mM ammonium formate with 0.1% formic acid in 95:5 methanol:water

Multisampler Pretreatment
• Improves the peak shape for early eluting polar compounds
• Match 10:1 (mobile phase A : sample)
RESIDUES BY LC-MS/MS

Calibration

• Matrix-matched calibration
  • Linear fit (min. 5 point) and quadratic (min. 6 point) fit

• Calibration range 0.2 ng/mL → 10 ng/mL

• 1/X weighting, exclude origin

• Correlation coefficient >0.990
Abamectin
$r^2=0.9974$

Acequinocyl-hydroxy
$r^2=0.9984$

Daminozide
$r^2=0.9929$
Spinosyn A  
$r^2=0.9979$

Spirotetramat  
$r^2=0.9991$

Spiroxamine  
$r^2=0.9978$
RESIDUES BY LC-MS/MS

50µL extract + 950µL acetonitrile

Final dilution factor of 20x for analysis
LC-MS/MS RECOVERIES

0.2 mg/kg
n=5

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<th>% RSD</th>
<th>Compound</th>
<th>% REC</th>
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<td>2.89</td>
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</table>
Abamectin, 1.0 ng/mL
Acequinocyl-hydroxy, 0.5 ng/mL
Daminozide, 1.0 ng/mL
Spinosyn A, 0.2 ng/mL
Spirotetramat, 0.2 ng/mL
Spiroxamine, 0.2 ng/mL
RESIDUES BY LC-MS/MS

Evaluation of cleanups

• Universal (PSA / C18 / GCB / MgSO$_4$)
• C18 / MgSO$_4$
• C18 / PSA / MgSO$_4$
RESIDUES BY LC-MS/MS

Evaluation of cleanups

- Universal (PSA / C18 / GCB / MgSO₄)
- C18 / MgSO₄
- Fatty (C18 / PSA / MgSO₄)

- Excellent matrix removal
- Poor compound recovery
  - Addition of toluene, hexane or acetone will require solvent exchange
  - Adds additional preparation steps
RESIDUES BY LC-MS/MS

Evaluation of cleanups

- Universal (PSA / C18 / GCB / MgSO₄)
- C18 / MgSO₄
- C18 / PSA / MgSO₄

- Excellent matrix removal
- Poor compound recovery
  - Addition of toluene, hexane or acetone will require solvent exchange
  - Adds additional preparation steps
RESIDUES BY LC-MS/MS

Evaluation of cleanups

• Universal (PSA / C18 / GCB / MgSO₄)
• C18 / MgSO₄ • Adequate matrix removal
  • Good compound recovery
• C18 / PSA / MgSO₄
RESIDUES BY LC-MS/MS

Evaluation of cleanups

- Universal (PSA / C18 / GCB / MgSO₄)
- C18 / MgSO₄
- C18 / PSA / MgSO₄

- Adequate matrix removal
- Good compound recovery
RESIDUES BY LC-MS/MS

Evaluation of cleanups

- Universal (PSA / C18 / GCB / MgSO₄)
- C18 / MgSO₄
- C18 / PSA / MgSO₄

- Excellent matrix removal
- Poor recovery for some compounds
  - Daminozide
  - Spinosad
  - Spirotetramat
  - Spiroxamine
RESIDUES BY LC-MS/MS

Evaluation of cleanups

• Universal (PSA / C18 / GCB / MgSO₄)
• C18 / MgSO₄
• C18 / PSA / MgSO₄

Can we improve the recoveries without sacrifice to the cleanup benefits of PSA?

• Excellent matrix removal
• Poor recovery for some compounds
  • Daminozide
  • Spinosad
  • Spirotetramat
  • Spiroxamine
Methanol can be added at d-SPE step to improve the recoveries of problematic compounds.

Determine methanol addition percentage to optimize compound recovery with minimal effects of PSA matrix removal.

**Daminozide cannot be recovered from PSA using this technique.**
RESIDUES BY LC-MS/MS

Response as a Function of % Methanol

<table>
<thead>
<tr>
<th>Compound</th>
<th>50% MeOH</th>
<th>10% MeOH</th>
<th>Acetonitrile</th>
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</thead>
<tbody>
<tr>
<td>Spiroxamine</td>
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<tr>
<td>Spirotetramat</td>
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<tr>
<td>Spinosyn A</td>
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RESIDUES BY LC-MS/MS

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RESIDUES BY LC-MS/MS

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Dispersive cleanup and dilution

500µL extract + 300µL methanol + 200µL acetonitrile into 2mL d-SPE (30% MeOH)

Vortex 30 sec
Centrifuge 2 min

100µL extract
900µL acetonitrile

Agilent Fatty Samples d-SPE (5982-5122)
- 50 mg PSA
- 50 mg C18EC
- 150 mg MgSO₄
Dispersive cleanup and dilution

500µL extract + 300µL methanol + 200µL acetonitrile into 2mL d-SPE (30% MeOH)

Vortex 30 sec
Centrifuge 2 min

Final dilution factor of 20x for analysis

100µL extract 900µL acetonitrile
Summary

- JASBC extraction results in clean initial sample extract

- DILUTION → can be a more powerful tool than d-SPE techniques alone, even better when combined
  - Sample prep plus extract dilution gives a 500x total sample dilution at the instrument

- Cleanup techniques tailored to specific analytical techniques
  - GC-MS/MS → Universal (PSA / C18 / GC / MgSO₄)
  - LC-MS/MS → Fatty (C18 / PSA / MgSO₄) with methanol assist
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