The Determination of THC-COOH (THCA) in Hair using GC-GC-MS

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Marijuana
- THC is active ingredient
- Major urinary metabolite is THCA
- Parent THC is detected in higher concentrations in hair than THCA
- However, to avoid environmental contamination concerns in workplace samples, detection of the metabolite THCA is required

Objective
- Proposed Federal Guidelines:
  Cut-off for THCA in hair: 0.05 pg/mg
- Aim:
  - To achieve this sensitivity using a single quadrupole mass spectrometric system, producing at least two ions for identification

Department of Health and Human Services. Substance Abuse and Mental Health Services Administration. Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug testing Programs. Federal Register 69(71); 2004

Literature

<table>
<thead>
<tr>
<th>Author</th>
<th>Methodology</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kintz, et al. J Forensic Sci. 40(4): 619-22 (1995)</td>
<td>100 mg sample NaOH digestion L/L extraction NCI GC/MS Reagent gas: methane</td>
<td>LOD: 5 pg/mg Mean THCA level from users: 0.12 ng/mg (2400 x higher than proposed cut-off)</td>
</tr>
</tbody>
</table>

Literature (continued)

<table>
<thead>
<tr>
<th>Author</th>
<th>Methodology</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore, et al. J Anal Toxicol. 25(7): 555-59 (2001)</td>
<td>NaOH digestion SPE extraction NCI GC/MS Reagent gas: methane</td>
<td>LOD 0.5 pg/mg (10 x higher than proposed cut-off)</td>
</tr>
<tr>
<td>Sachs and Dressler Forens Sci Int 107(1-3): 239-247 (2000)</td>
<td>Clean-up using HPLC NCI GC/MS Reagent gas: methane</td>
<td>LOD 0.3 pg/mg</td>
</tr>
<tr>
<td>M. Uhl. Forens Sci Int 107(1-3): 169-79 (2000)</td>
<td>GC/MS/MS</td>
<td>LOD 0.16 pg/mg</td>
</tr>
</tbody>
</table>
Approach

Small improvements at all stages of the procedure:

1. Extraction
2. Gas Chromatography
3. Mass Spectrometry

1. Extraction

A. Sonication: Methanol:EtAc (50:50 v:v; room temp; 2 hrs)
   • 50% recovery, clean

B. Digestion: NaOH; Liquid-liquid extraction (hexane:EtAc)
   • Improved recovery; dirty extract

Extraction (continued)

C. Acetone wash; digestion (NaOH); SPE: strong anion exchange column (SPEWare PolyChrom THC 682-0353)
   • Good extraction recovery, dirty extract

D. Methylene chloride wash; digestion (NaOH); neutralization, SPE: cation exchange/hydrophobic column
   • Best results, full method described

Extraction Procedure

Standards:

- Tri-deuterated THCA (100 µg/mL)
- THCA (1 mg/mL)
  - Cerrilliant (Round Rock, TX)

- Internal standard concentration: 1 pg/mg

Extraction Procedure (continued)

Calibrators, controls, specimens: 20 mg hair
Cut, weigh into tapered bottom glass tubes
Rinse: methylene chloride (1.5 mL)
Decant solvent; allow hair to dry
Add internal standard (1 pg/mg D3-THCA)
Calibration curve:
  • Negative, 0.05, 0.1, 0.5, 1.0, 5.0 pg/mg

Extraction Procedure (continued)

Add DI water (0.5 mL); 2N NaOH (0.5 mL)
Heat 75°C, 15 min
Cool; centrifuge (2500 rpm; 15 min)
Pour supernatant into glass tubes already containing:
  • Acetic acid (1 mL)
  • 1 M acetic acid (3 mL)
  • 0.1 M sodium acetate buffer (pH 4; 2 mL)
Solid Phase Extraction Procedure

- Condition SPE columns (hydrophobic/cation exchange):
  - Hexane:EtAc (75:25 v,v 2 mL)
  - Methanol (3 mL)
  - DI water (3 mL)
  - 0.2M hydrochloric acid (1 mL)
- Load samples
- Wash
  - DI water (2 x 3 mL)
  - 0.1 M HCl:acetonitrile (70:30 v,v 3 mL)

Extraction Procedure (continued)

- Elute THCA:
  - Hexane:EtAc (75:25, v,v 3 mL)
- Evaporate eluent to dryness (N₂; 40°C)
- Reconstitute:
  - Trifluoroacetic anhydride (TFAA, 50 µL)
  - 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 30 µL)
- Cap, heat (70°C/15 min)
- Leave at room temp for 10 min
- Evaporate to dryness (vacuum oven)
- Reconstitute in toluene (25 µL)

2. Gas Chromatography

A. Two-dimensional GC (GC:GC)
- Serial columns
- As different in polarity as possible
- Dean’s switch allows flow to be diverted from primary column to analytical column

Deans’ Switch system:
Used to divert analyte of interest from DB-35MS to DB-1MS

Switch off:
DB-35MS effluent goes to FID (no cut)

Switch on:
DB-35MS effluent is cut to DB-1MS
2. Gas Chromatography (continued)

B. Cryogenic focusing

- Used to “cold trap” the analyte as it passes through analytical column
- Operated through “Back Inlet” software of system
- Cooled using compressed air

3. Mass Spectrometry

- Derivatization:
  - Maximize number of electronegative, volatile groups on molecule because of selected mode
- Tried various combinations:
  - HFIP/PFPA; PFPAH/PRTA; HFBA/PFPA; TFAA/PFPA; TFAA/HFIP
  - Trifluoroacetic anhydride adds COCF₃ (97 mass units)
  - 1,1,1,3,3,3-hexafluoro-2-propanol adds CH(CF₃)₂ (151 mass units)
3. Mass Spectrometry (continued)

- **Instrument parameters:**
  - **Reagent gas:**
    - Ammonia (~7 x more sensitive than methane)
    - Purity 99.999% with max impurities:
      - CO 1 ppm; Methane 1 ppm; N₂ 1 ppm;
      - O₂ 1 ppm; H₂O 5 ppm
    - Stainless steel regulator
  - **Mode:**
    - Negative ion chemical ionization (NCI)
    - Electron Capture Chemical Ionization (ECCI)

- **Collision gas flow:** $8 \times 10^{-5} – 1.0 \times 10^{-4}$ Torr
- **MS Source:** 150°C
- **MS Quadrupole:** 106°C
- **Transfer line:** 280°C
- **SIM ions:** 590 (593); 422 (425)
- **Retention Time:** 13.92 min

**Hair specimen:** Negative
Hair specimen: 1.21 pg/mg THCA

THCA detected in hair of self-reported marijuana users

Summary
- Procedure makes use of minor modifications in methodology:
  - Cleaner extraction
  - Efficient derivatization
  - Minimal background from the matrix diverted to analytical column
  - Cryogenic focusing
  - NCI mode using ammonia as reagent gas improves sensitivity

Conclusion
- Described method uses single quadrupole mass spectrometer to routinely achieve Federally proposed concentrations for THCA in hair

### Table

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>GC/MS/MS</th>
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<th>Self-reported Frequency</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1.98</td>
<td>1.94</td>
<td>No use</td>
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<tr>
<td>4</td>
<td>0.49</td>
<td>0.55</td>
<td>2 x week</td>
</tr>
<tr>
<td>5</td>
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<td>0.24</td>
<td>3 x week</td>
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<tr>
<td>2</td>
<td>1.66</td>
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<td>7 x week</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>12</td>
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<td>56 x week</td>
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<tr>
<td>MEAN</td>
<td>0.899</td>
<td>0.922</td>
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