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TITLE

THC and THC-COOH, GC/MS, Blood

REFERENCES

9. “Reagent Chemicals” American Chemical Society Committee on Analytical Reagents, Washington, DC.

METHOD

Gas chromatography/mass spectrometry.

PRINCIPLE

THC and its major metabolite, 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid (Δ-9-THC-COOH), are readily analyzed in blood specimens. The metabolite may be present in blood in the unconjugated form. An internal standard solution containing deuterated THC-D3 and THC-COOH-D9 is added to a blood that has screened positive. One milliliter of blood is precipitated and extracted with hexane/ethylacetate, centrifuged, and evaporated to dryness. The extract is then derivatized by bis-(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane to form the trimethylsilyl derivatives, which are analyzed by mass
spectrometry. The mass spectrometer is operated in the selected-ion-monitoring (SIM) mode. For THC, three characteristic ions are monitored: \( M/z = 386, 315 \) and \( 303 \) and for the deuterated internal standard, the \( m/z = 389 \) and \( 318 \) ions are monitored. For THC-COOH, three characteristic ions, \( m/z = 488, 473, \) and \( 371 \), are selected for monitoring and the \( m/z = 491 \) and \( 476 \) ion peaks are selected for monitoring the deuterated internal standard.

**SAMPLE**

Blood, containing sodium fluoride (NaF), preservative, and potassium oxalate, anticoagulant.

**SAFETY**

Use routine precautions found in the Chemical Hygiene Plan (Appendix I – Safety Manual) when working in the laboratory. Follow the Bloodborne Pathogens Exposure Control Plan (Appendix G – Safety Manual), when working with biological fluids or tissues. Read all Material Safety Data Sheets before handling unfamiliar reagents.

**EQUIPMENT:**

1. Multiprep workstation: vacuum box, elution rack, and sample evaporator.
2. Adjustable volume pipettes.
4. Microliter syringes (Hamilton or equivalent).
5. Graduated cylinders.
7. Beakers.
8. Dry-bath incubator.
10. pH Paper.
11. 16x100 mm silanized borosilicate glass disposable culture tubes.
12. Crimp top vials with 0.25 ml, or 0.10 ml inserts.
13. Crimp caps 11 mm with teflon coated red silicone rubber septa.

15. GCMSD system (Or equivalent):
   a) HP 6890 GC with a fused silica column with a stationary phase of crosslinked methylsilicone - either 5% or 50%.
   b) HP 5975 mass selective detector - (MSD)
   c) HP 7683B automatic liquid sampler - (ALS)

REAGENTS

Reagent grade chemicals are used in all tests unless otherwise indicated. It is intended that all reagents will conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

Chemicals

1. Acetonitrile.

2. Concentrated Ammonium Hydroxide (NH₄OH).

3. Concentrated Hydrochloric Acid (HCl).

4. Hexane.

5. Ethyl acetate - (EA).


Gases

1. Helium gas tank, 99.999% purity.

2. Nitrogen gas tank, ultra high purity.

Derivatizing Agents

1. Bis-(trimethylsilyl) trifluoroacetamide with 1% TMCS- (BSTFA).
Controls

1. Negative Defibrinated sheep’s blood, purchased commercially.

2. Commercially available THC and THC-COOH purchased from a lab independent from which the calibration standards were purchased. A high and low level will be run with each run.

Standards/Internal Standards

1. THC-D3.
2. THC, (THC-D0).
4. THC-COOH, (THC-COOH-D0).
5. Negative Blood, 1% sodium fluoride.

REAGENT PREPARATION

Note: All “working” chemical solutions, mixtures, or dilutions shall be labeled with the following information, chemical name, concentration, date prepared, analysts’ initials, special storage instructions, and expiration date.

Stock Standard Preparation:

THC (1 mg/mL); Cerilliant Corporation, in sealed ampules containing 1 mg/ml of Delta-9-THC in 1 ml methanol.

THC-COOH (100 ug/ml); Cerilliant Corporation, in sealed ampules containing 100 ug/ml of 11-nor-∆9-THC-COOH in 1 ml methanol.

1. THC, (THC-D0), 100 ug/ml

Using a 1.0 ml syringe, transfer 1.0 ml of THC-D0 to a 10 mL volumetric flask; bring to volume with methanol. Transfer to a storage bottle. Cap and store in freezer (less than -4°C). Expiration date of 1 year.

2. Stock Standard Mix, THC and THC-COOH, 10 ug/ml

Using a 1.0 ml syringe, transfer 1.0 ml of Cerilliant Stock,THC-COOH and 1.0 ml of THC, 100 ug/ml stock, to a 10 mL volumetric flask; bring to volume with methanol. Transfer to a
storage bottle. Cap and store in freezer (less than -4°C). Expiration date of 1 year.

**Working Standard Preparation:**  
500 ng/ml THC-D0, and 500 ng/ml THC-COOH-D0.

1. Place exactly 0.5 ml of the Stock Standard Mix, 10 µg/ml into a 10 ml volumetric flask. Dilute to mark with methanol. Stable for 1 year at less than -4°C.

**Stock Internal Standard Preparation:**  
ThC-D3 (100 µg/ml); Cerilliant Corporation, in sealed ampules containing 100 µg/ml delta-9-THC-D3 in 1 ml methanol.

THC-COOH-D9 (100 µg/ml); Cerilliant Corporation, in sealed ampules containing 100 µg/ml of 11-nor-∆9-THC-COOH-D9 in methanol.

**Working Internal Standard Preparation:**  
1 µg/mL THC-D3 and 1 µg/ml THC-COOH-D9.

1. **Stock Internal Standard Mix, THC-D3 and THC-COOH-D9, 10 µg/ml**  
Transfer exactly 1.0 ml of each Stock Internal Standard from Cerilliant Corportation into a 10 mL volumetric flask. Dilute to mark with methanol. Expiration date of 1 year at less than -4°C.

2. **Working Internal Standard Mix, THC-D3 and THC-COOH-D9, 1 µg/ml**  
Transfer exactly 1.0 ml of the Stock Internal Standard Mix into a 10 mL volumetric flask. Dilute to the mark with methanol. Expiration date of 1 year at less than -4°C.

**Negative blood, 1% sodium fluoride**

Place 1 gram of sodium fluoride in a 100 ml volumetric flask. Fill to mark with defibrinated sheep’s blood and mix thoroughly. Blood must reach room temperature before use, to ensure proper percentage of sodium fluoride. Label and refrigerate. Expiration date of 6 months.

**1 N HCl**

Partially fill a 100 ml volumetric flask with distilled water. Add 8.2 mls of concentrated HCl. Fill to mark with distilled water.

**Control Preparation**

1. **Negative control:** Negative blood, 1% sodium fluoride.

2. **Positive controls:** A commercial NIST traceable controls are used. Purchased from a
manufacturer separate from the one used for standards if available. Controls are made in a similar manner to standards.

**SPECIMEN PREPARATION**

1. Obtain the test specimens/aliquots and working standards from refrigerated storage. Allow to reach room temperature before use.

2. Obtain negative sheep blood for preparing the calibration standards and controls, and unknown samples to be run from refrigerated storage. Place on rocker and mix for at least 10 minutes.

3. Label 5 tubes as follows and place the appropriate amount of negative blood and working standard mix into each calibration standard.

   a. 5 ng/ml Standard.
      0.990 ml negative blood
      10 ul working standard mix

   b. 10 ng/ml Standard.
      0.980 ml negative blood
      20 ul working standard mix

   c. 25 ng/ml Standard.
      0.950 mls negative blood
      50 ul working standard mix

   d. 50 ng/ml Standard.
      0.900 mls negative blood
      100 ul working standard mix

   e. 100 ng/ml Standard
      0.800 mls negative blood
      200 ul working standard mix

   Appropriately label a second set of tubes for each of the Calibration Standards, the negative and positive controls, and the test specimens in the run. Set aside for future use.

4. Place 1 ml of each negative and positive controls as well as test specimens in appropriately labeled tubes.

**NOTE: NEVER RETURN ANY PORTION OF THE SPECIMEN, ONCE REMOVED, TO THE ORIGINAL CONTAINER OR INSERT ANYTHING OTHER THAN A NEW OR CLEAN PIPETTING DEVICE INTO THE SPECIMEN.**
5. Add 100 ul of working internal standard solution to each tube.

6. Vortex samples.

EXTRACTION

1. Add 2.0 mls of acetonitrile to each tube. Vortex samples until well mixed.

2. Centrifuge samples for 5 minutes at 4000 rpm.

3. Decant supernatant into new, appropriately labeled tubes.

4. Add 100 ul of concentrated ammonium hydroxide and vortex samples.

5. Dry down the samples for 20 minutes with air or nitrogen.

6. Add 2 ml of 1 N HCl and vortex until well mixed.

7. Add 3 mls of hexane/ethyl acetate (9:1) extraction solvent to each tube. Cap tubes and mix by gentle inversion for at least 3 minutes.

8. Centrifuge for 2 minutes @ 3000 rpm. Transfer the top layer to a clean glass tube.

CONCENTRATION

1. Evaporate the solvent to dryness under a stream of nitrogen or air.

DERIVATIZATION

CAUTION - In the event of a spill, absorb the derivatizing agent with vermiculite. Place in a small autoclave bag, and seal with tape. This may then be disposed of in the normal trash. Ventilate area and wash spill site after material has been cleaned up.

1. When tubes are completely dry, add 35 ul of ethyl acetate and 35 ul of BSTFA (with 1% TMCS) to each tube.

2. Cap tubes and place them back into the water bath or heating block at 70-80 °C for 20 minutes to allow for derivatization.

3. Using a micropipette, transfer the remaining solution into 100ul inserts in appropriately labeled crimp top GC/MS sample vials.

4. Cap samples using crimping tool. Make sure vials are tightly sealed.
ANALYSIS

Inject 1 ul of the remaining BSTFA solution into the GC/MS using the established SIM method for this analysis.

Note: Do not contaminate with water or methanol; these substances destroy the trimethylsilyl derivative and will react with BSTFA in the sample tubes.

GC/MS Parameters

Current GC/MS method parameters are located in the GC/MS Methods Logbook and are automatically loaded into the instrument when the sequence runs.

MSD SIM Program

<table>
<thead>
<tr>
<th>Drug</th>
<th>BSTFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC-D3</td>
<td>389 and 318</td>
</tr>
<tr>
<td>THC</td>
<td>386, 315, and 303</td>
</tr>
<tr>
<td>THC-COOH-D9</td>
<td>491 and 476</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>488, 473, and 371</td>
</tr>
</tbody>
</table>

Instrument Set Up

1. Perform routine maintenance and autotune as described in the Tuning and Troubleshooting an Agilent 597X MSD Standard Operating Procedure.

Operating Temperatures:*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Temperature</td>
<td>180°C</td>
</tr>
<tr>
<td>Initial Time</td>
<td>1.0 min</td>
</tr>
<tr>
<td>Rate</td>
<td>10°C/ min</td>
</tr>
<tr>
<td>Final Temperature</td>
<td>290°C</td>
</tr>
<tr>
<td>Final Time</td>
<td>10.0 min</td>
</tr>
<tr>
<td>Injector Port A</td>
<td>250°C</td>
</tr>
<tr>
<td>Detector A</td>
<td>280°C</td>
</tr>
<tr>
<td>Transfer Line</td>
<td>280°C</td>
</tr>
</tbody>
</table>

* Operating conditions, e.g. temperatures, column length, etc. may be adjusted to optimize the assay.

Injection Sequence

THC 10 ng neat (for setting retention times)
THC 5 ng standard
THC 10 ng standard
THC 25 ng standard
THC 50 ng standard
THC 100 ng standard
THC Positive Control Low
THC Positive Control High
WASH
THC Negative Control
Specimens (perform WASH between each specimen)
END THC 10 ng standard
END THC Positive Control High
END THC Negative Control

DATA INTERPRETATION AND QUALITY CONTROL

1. Perform calibration, data analysis, and any data reprocessing that might be required following the MS Chemstation User’s Guide, HP G1034C Ms Chemstation Software, Hewlett-Packard Company.

2. Record all quality control results in the Quality Control Logbook.

3. Assemble a data package containing initial calibration information, quantitation reports, chromatograms for all standards, controls and samples and correction action logs and submit to the Quality Control Analyst for review and the Toxicology Supervisor or designee for approval in accordance with the Toxicology Data Review, Approval, and Reporting Instruction.

PROCEDURE NOTES AND LIMITATIONS OF METHOD

1. Linearity: 5 ng/ml to 100 ng/ml.

2. Specificity: The retention time, ion ratios and deuterated internal standards define the specificity for this analysis. Also, the specificity can be calculated from the relationship: True Negatives / (True Negatives + False Positives). This relationship provides a specificity of less than 100% only when a false positive is encountered with the negative control. If the negative control is always found to be negative, the specificity for the analysis is 100%.

3. Relative Recovery: Absolute recovery is not a requisite due to the use of the isotopic dilution with deuterated internal standards.

4. L.O.D. (Limit of Detection) and L.O.Q (Limit of Quantitation): The limit of detection and quantitation were established by analyzing 8 or more specimens known to be negative for THC. The background counts for the m/z = 386 and the 488 ion over the time range of ± ½ minute of the mean of the retention times for the standards that were used to produce the calibration curve were determined. The background count divided by the m/z = 389
and the 497 ion respectively, area abundance counts at that retention time produced a ratio that was compared to the low calibration standard. A numeric value was fixed to the ratio. The mean and standard deviation of the 10 negative samples were calculated.

The Limit of Detection (L.O.D.) = mean + 3 S.D

\[
\begin{align*}
\text{THC} &= 1.6 \text{ ng} \\
\text{THC-COOH} &= 3.8 \text{ ng}
\end{align*}
\]

The Limit of Quantitation (L.O.Q) = mean + 10 S.D.

\[
\begin{align*}
\text{THC} &= 3.0 \\
\text{THC-COOH} &= 4.9
\end{align*}
\]

6. Interfering Substances: No known interferences have been noted to date.

7. Validation of the 5 ng/ml Cut-Off Concentrations: The procedure was performed in 10 replicates of a spiked standard of 5 ng/ml. The concentration was calculated using the calibration curve. The standard deviation was calculated to be 0.90 ng/ml for THC and 1.07 for THC-COOH.

8. Carryover: Following injection of an extract containing 500 ng/ml, three negative controls were found to be qualitatively negative.

9. Chemicals prepared fresh on the day of use are determined to be acceptable on the basis of an acceptable performance of the positive and negative controls.

10. THC is extensively protein bound and poorly distributed to red cells, the concentration of THC in whole blood is approximately half that found in serum or plasma. However, blood containing sodium fluoride can be used and is preferred when serum or plasma are not available.

**POLLUTION PREVENTION**

1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in a laboratory operation. The United States Environmental Protection Agency (US EPA) has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot feasibly be reduced at the source, the US EPA recommends recycling as the next best option.

2. The quantity of chemicals purchased should be based on expected usage during the shelf life
and disposal cost of unused material. Actual reagent preparation volume should reflect anticipated usage and reagent stability.

3. For information about pollution prevention that may be applicable to laboratories, consult “Less is Better: Laboratory Chemical Management for Waste Reduction”, available from the American Chemical Society’s (ACS) Department of Governmental Regulations and Science Policy, 1155 16th Street NW, Washington D.C. 20036, (202) 872-4477.

WASTE MANAGEMENT

1. The US EPA requires that laboratory waste management practice be consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the “Waste Management Manual for Laboratory Personnel”, available from The American Chemical Society at the address listed above.

2. Dispose of all consumables contaminated by blood in appropriate biohazard waste containers for autoclaving.

3. Contact the Chemical Hygiene Office for disposal recommendation of other chemicals or solutions.
### Revisions

**File Name:**  
**Revision:**  
**Date:**  

**Edited by:**  
**Date:**  

**Section Supervisor:**  
**Date:**  

**Program Manager:**  
**Date:**  

**Quality Assurance Officer:**  
**Date:**  

**Division Director:**  
**Date:**  

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