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2.4.4 Qualitative 11-nor-9-THC- Δ^9 -COOH (Carboxy-THC)

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Section Two

Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

2.4.4 Qualitative 11-nor-9-THC- Δ^9 -COOH (Carboxy-THC)

2.4.4.1 BACKGROUND

Cannabis sativa use dates back to 2700 B.C.^{2,5} Marijuana refers to a mixture of the leaves and flowering tops.³ The smoke from burning cannabis includes 61 different cannabinoids.^{2,6} The major active ingredient in marijuana is delta-9-tetrahydrocannabinol (Δ^9 -THC). The Δ^9 -THC content varies from 2 to 10% with an average of four to five percent. The quality of marijuana is reported to have improved over the last 20 years due to superior cultivation practices. The medicinal effects of marijuana include antinausea, muscle relaxing, anticonvulsant and reduction of intraocular pressure.⁶ Cannabis therefore has found use as an antiemetic to deal with the nausea associated with anticancer chemotherapy and for relief for those suffering from glaucoma. The debate continues on medical use and the complete legalization of the drug.

Several factors come into play when considering the behavioral effects of (Δ^9)-THC. These include the route of administration (smoked or ingested), THC concentration of the plant (dose), the experience of the user, the user's vulnerability to psychoactive effects, and the setting of the use.^{5,6} The desirable effects of marijuana include an increased sense of well-being, mild euphoria, relaxation and a mild sedative-hypnotic effect.^{5,6} Its clinical effects are similar to those of alcohol and the antianxiety agents.⁵ The side-effects of marijuana use include impairment of cognitive functions, alteration of the user's perception of time and distance, reaction time, learning and short-term memory.^{2,5,6} Marijuana has been shown to interfere with a person's ability or willingness to concentrate. Cannabis causes temporal disintegration such that the individual loses the ability to store information in the short term and is easily distracted.² Impairment from use is thought to last from 4 to 8-hours with more recent studies reporting 3 to 6 hours. Dr. Huestis reported that most behavioral and physiological effects return to baseline within three to six hours after use with residual effects in specific behaviors for up to 24 hours.

Impairment of coordination and tracking behavior has been reported to persist several hours beyond the perception of the high.⁶ Due to the variable period of impairment the relating of urine carboxy-THC to the time of use, and thus impairment, requires the development of the scenario surrounding the stop for DUI. The presence of carboxy-THC in urine only indicates exposure to MH at some previous indeterminate time.

The physiological effects may include an increase in heart rate and blood pressure, conjunctival suffusion, vasodilation, dry mouth and throat and a decrease in respiratory rate. The individual may also experience increased hunger (munchies).

Δ^9 -THC is rapidly metabolized to the inactive metabolite, carboxy-THC.^{1,4,5,6} In urine, this major metabolite, carboxy-THC is pursued due to Δ^9 -THC only being present in minute quantities.⁶ Carboxy-THC in urine has been conjugated with glucuronic acid to improve excretion. The detection time of carboxy-THC in urine following marijuana use varies dependent upon various pharmacological factors such as the dose obtained, the route of administration and the rates of metabolism and excretion.¹ Δ^9 -THC is deposited in body fat due to its high lipid solubility. It is slowly released from this storage depot over time.¹ The amount of Δ^9 -THC stored in fat is a function of the amount, frequency and potency of drug exposure. The detection time can therefore vary from days to months.

2.4.4.2 SCOPE

This method is to qualitatively confirm the presence of a major metabolite of marijuana, carboxy-THC, in urine specimens. Samples are subjected to an alkaline hydrolysis to liberate carboxy-THC from its glucuronide conjugate. Hydrolyzed samples are then made acidic with a phosphate buffer and extracted with hexane/ethyl acetate 87:13. Following centrifugation the extract is removed and dried under nitrogen. The dried extract is silylated to form a TMS derivative. The derivative is analyzed on a GC/MSD in SIM mode.

2.4.4.3 EQUIPMENT AND SUPPLIES

- 2.4.4.3.1 Tube Rocker
- 2.4.4.3.2 Laboratory Centrifuge
- 2.4.4.3.3 Waterbath
- 2.4.4.3.4 Drybath
- 2.4.4.3.5 Evaporative Concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.
- 2.4.4.3.5 pH Indicator Paper Strips
- 2.4.4.3.6 Glassware
 - 16X100mm tubes
 - 16X144mm tapered tip centrifuge tubes (optional)
 - Snap caps for 16mm OD tubes (optional)
 - GC/MS ALS vials
 - GC/MS vial microinsert
- 2.4.4.3.7 Gas Chromatograph equipped with a mass selective detector and a nonpolar capillary column (e.g. 100%-dimethylpolysiloxane or 95%-dimethylpolysiloxane with 5% diphenyl).

2.4.4.4 REAGENTS

Refer to manual section 5.12 for solution preparation instructions. Purity of chemicals must be ACS Grade or equivalent.

- 2.4.4.4.1 1.0 N KOH
- 2.4.4.4.2 Saturated Potassium Phosphate Monobasic pH \approx 1.8
- 2.4.4.4.3 87:13 Hexane with Ethyl Acetate (v/v)
- 2.4.4.4.4 Ethyl acetate
- 2.4.4.4.5 Silylating Agent (select from)
BSTFA/1% TMCS or MSFTA

2.4.4.5 STANDARDS

- 2.4.4.5.1 Stock Standard Solution
 - 2.4.4.5.1.1 100 μ g/mL (+) 11-nor-9-carboxy- Δ^9 -THC (Radian T-006 or equivalent).
- 2.4.4.5.2 Working Standard Solution (1800ng/mL)
 - 2.4.4.5.2.1 Add 900 μ L Stock Solution to 49.1mL Methanol. Solution is stable for six months when stored at 4°C.

2.4.4.6 QUALITATIVE CONTROLS

- 2.4.4.6.1 Liquid Urine Control containing Carboxy-THC
- 2.4.4.6.2 60ng/mL Carboxy-THC Positive Control Urine
Add 3mL of the same lot of negative urine used to prepare the negative control to an extraction tube. Add 100 μ L of working standard solution, and vortex.
- 2.4.4.6.3 Extracted Negative Control
Negative urine commercially obtained or in-house urine verified to be negative for drugs of interest.

2.4.4.7 PROCEDURE

- 2.4.4.7.1 Initial set-up
Label extraction tubes, tapered bottom derivatization tubes and GC/MS vials with microinserts as follows for the negative control (NC), positive control (PC), Liquid Control and appropriate laboratory numbers without prefix.
- 2.4.4.7.2 Sample Preparation
Transfer 3 mL of urine specimen, negative urine, positive control and liquid control to extraction tube.
- 2.4.4.7.3 Sample Hydrolysis
 - Add 0.5mL of 1.0N KOH to each extraction tube.
 - Vortex *gently* to mix.

- Check resulting pH with pH indicator strip.
- pH must be ≥ 12 . If <12 add an additional 0.5mL of KOH.
- Place in 40°C waterbath for 15 minutes.
- Allow samples to cool before proceeding with solvent extraction.

2.4.4.7.4 Extraction

If original pH was @ ≥ 12 :

- Add 1.5mL of phosphate buffer.
- Add 3mL of Hexane/Ethyl Acetate (87:13).
- Rock for 10 minutes.

If original pH was @ <12 :

- Add 3.0mL of phosphate buffer.
- Add 4mL of Hexane/Ethyl Acetate (87:13).
- Rock for 15 minutes.

2.4.4.7.5 Centrifuge tubes at 3500 rpm for 10 minutes.

2.4.4.7.6 Transfer upper organic phase from tube into labeled tapered bottom tube.

2.4.4.7.7 Evaporate solvent to dryness, under a gentle stream of nitrogen, in Turbo Vap at 37°C.

2.4.4.7.8 Derivatization

- To dried extract in tapered bottom tubes, add 50 μ L ethyl acetate and 50 μ L silylating reagent.
- Cap tubes with snap caps.
- Vortex.
- Heat tube for 15 minutes in 95°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MS ALS vial with microinsert.

2.4.4.8 **GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)**

2.4.4.8.1 Preparation for Analysis Run

2.4.4.8.1.1 Into Sequence log table, enter the sample case numbers, blanks and controls.

2.4.4.8.1.2 Load samples, reference material, blank and controls into the quadrant rack as noted in the sequence table.

2.4.4.8.2 GC-MSD Analysis Parameters

2.4.4.8.2.1 Refer to instrument METHOD for current analysis parameters.

2.4.4.8.2.2 Current analysis method must be stored centrally as a hard or electronic copy.

2.4.4.8.2.3 Analyze sample extract in SIM (selected ion monitoring) utilizing the ions 371, 473 and 488.

2.4.4.8.3 Detection and Identification Criteria**2.4.4.8.3.1** **Retention Time**

Identification requires a peak within ± 0.2 minutes of the run retention time established for carboxy-THC.

2.4.4.8.3.2 **Ion ratios - Qualitative Selective Ion Monitoring (SIM)**

Ion ratio for pre and post-run 60ng carboxy-THC standards should be established. This ratio should be compared to ratio obtained from casework sample. Ratio between monitored ions, 371, 473 and 488, should agree within $\pm 20\%$.

2.4.4.9 **QUALITY ASSURANCE REQUIREMENTS****2.4.4.9.1** General

2.4.4.9.1.1 Urine samples are to be stored frozen until allowed to thaw prior to analysis.

2.4.4.9.1.2 Urine samples are to be stored under refrigeration after aliquots are removed for analysis.

2.4.4.9.1.3 Post analysis, urine samples are to be stored frozen until appropriate disposal date.

2.4.4.9.1.4 Refer to toxicology analytical methods 5.8 and 5.10 for additional quality assurance and reference material authentication requirements.

2.4.4.10 **ANALYSIS DOCUMENTATION**

2.4.4.10.1 Original data for controls will be compiled for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

- 2.4.4.10.2 A copy of controls need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

2.4.4.11 REFERENCES

- 2.4.4.11.1 Huestis, M.A., Mitchell, J.M. and Cone, E.J. *Detection Times of Marijuana Metabolites in Urine by Immunoassay and GC-MS*. *J. Anal. Tox.* **19**:443-449, 1995.
- 2.4.4.11.2 Huestis, M. *Marijuana*. pp. 246-264. *in: Principles of Forensic Toxicology*. Levine, B. ed., AACC, 1999.
- 2.4.4.11.3 *Cannabis*. *in: Clark's Isolation and Identification of Drugs* pp. 423-425, Moffat, A.C. ed., Pharmaceutical Press:London, 1986.
- 2.4.4.11.4 Drug Evaluation and Classification Training Manual, U.S. Dept. of Transportation, 1993.
- 2.4.4.11.5 Julien, R.M. *Marijuana: A Unique Sedative-Euphoriant-Psychedelic Drug*. *in: A Primer of Drug Action*. pp. 319-349, W.H. Freeman and Company:New York, 1998.
- 2.4.4.11.6 O'Brien, C.P. *Drug Addiction and Drug Abuse*. pp. 572-573. *in: Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th edition, Hardman, J.G. ed., McGraw-Hill, 1996.

Revision History

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Revision No.	Issue Date	History
1	11-27-2001	Original Issue in SOP format
2	09-13-2002	Clarification of detection and identification criteria and refinements
3	05-07-2008	Updated QA references and language
4	07-28-2008	Clarified that negative urine used to prepare positive control is the same lot as used for negative control.

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