Idaho State Police
Forensic Services
Toxicology Section

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)

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2.4.4.1 BACKGROUND

Cannabis sativa use dates back to 2700 B.C.[2,5] Marijuana refer to a mixture of the leaves and flowering tops.[3] The smoke from burning cannabis includes 61 different cannabionoids.[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 marihuana 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 the 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), the 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 marihuana 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 marihuana 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 it's 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.

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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 (Fisher Scientific or equivalent)

2.4.4.3.2 Laboratory Centrifuge (Fisher Marathon or equivalent)

2.4.4.3.3 Waterbath (Fisher or equivalent)

2.4.4.3.4 Drybath (Fisher or equivalent)

2.4.4.3.5 Evaporative Concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.4.4.3.5 Whatman® pH Indicator Paper Strips (Fisher 09-876-17 or equivalent)

2.4.4.3.6 Glassware

16X100mm tubes (Fisher 14-959-35AA or equivalent)
16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)
Snap caps (Fisher 05-538-41N or equivalent)
GC/MS ALS vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.4.4.3.7 Gas Chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%diphenyl).

2.4.4.4 REAGENTS

Refer to manual section 2.6 for solution preparation instructions. Purity of chemicals should be certified ACS or equivalent.

2.4.4.4.1 1.0 N KOH

2.4.4.4.2 Saturated Potassium Phosphate Monobasic pH ≈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 (Pierce #38842ZZ or equivalent)
MSFTA (Pierce #48910 or equivalent)
BSTFA with 1%TMCS (Pierce #38831 or equivalent)

2.4.4.5 STANDARDS

2.4.4.5.1 Stock Standard Solution

2.4.4.5.1.1 100µg/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 900µL Stock Solution to 49.1mL Methanol. Solution is stable for six months when stored at 4°C.

2.4.4.6 CONTROLS

2.4.4.6.1 Liquid Urine Control containing Carboxy-THC (Utak 66814, 66825, 98816 or equivalent)

2.4.4.6.2 60ng/mL Carboxy-THC Positive Control Urine

Add 100µL of working standard solution to 3mL of negative urine and mix.

2.4.4.6.3 Negative Control Urine (FS Personnel).

2.4.3.7 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.

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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

Original pH @ ≥ 12

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

Original pH @ <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 TurboVap at 37°C.

2.4.4.7.8 Derivatization

- To dried extract in tapered bottom tubes, add 50µL ethyl acetate and 50µL silylating reagent (BSTFA or MSTFA).
- 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.

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2.4.3.8 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

2.4.4.8.1 Inject 1µL TMS derivative into GC/MS using the ALS.

2.4.4.8.2 Analyze sample extract in SIM (selected ion monitoring) utilizing the ions 371, 473 and 488. Refer to attached GC/MSD method printout for current analysis parameters.

2.4.4.8.2.1 Detection and Identification Criteria

The presence of a drug compound can be established if there are no significant differences in the retention time and selected ion ratios are ±20%.

- Acceptable retention time window is +/- 5%.

2.4.4.9 REFERENCES

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