Name of Procedure:

Toxicology
THC and THC-COOH Extraction Procedure Using United Chemical Technologies Clean Screen Extraction Columns®

Suggested Uses:

This procedure is an extraction of delta-9-tetrahydrocannabinol (THC) and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) from blood using United Chemical Technologies Clean Screen Extraction Columns®. The procedure is designed to extract THC and THC-COOH for confirmation by mass spectrometry. The procedure targets THC and THC-COOH in a 1.0 mL blood sample. Calibration standards may be utilized for quantitative analysis.

Items Used to Perform Procedure:

Test tubes, 16 x 125, 13 x 100, 12 x 75
Test tube caps or stoppers
Vortexer
Centrifuge
pH paper
pH meter
Eppendorf pipettes
Pipet tips
Sonicater
Volumetric flasks
World Wide Monitoring Clean Screen Extraction Columns® CSTHC203
Zymark RapidTrace SPE Workstation
Zymark TurboVap LV
Nitrogen at 30 psi

Reagents Used:

Acquired drug standards
- Delta-9-tetrahydrocannabinol (THC)
- 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)

Acquired deuterated drug standards
Delta-9-tetrahydrocannabinol (THC)-D_3
11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid-D_3
Deionized water
Methanol
Hexane
Ethyl Acetate
Acetonitrile
BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamine with 1% trimethylchlorosilane)

Hydrochloric acid, 0.1M

a. Add 4.2 mL concentrated hydrochloric acid to approximately 400 milliliters of deionized water.
b. Dilute to 500 mL with deionized water. Mix.
c. Label with expiration date. Stability: 6 months at room temp.

Sodium Acetate buffer, 0.1M, pH 4.5

a. Dissolve 2.93 g of Sodium acetate trihydrate in 400 mL deionized water.
b. Add 1.62 mL of glacial Acetic Acid.
c. Dilute to 500 mL with deionized water.
d. Adjust pH to 4.5 +/- 0.1 with 100 mM sodium acetate or 100 mM acetic acid.
e. Label with expiration date. Stability: 6 months at room temp.

Internal Standard Solution

a. Internal standard: Prepare an internal standard solution that contains 1.0 microgram/mL (1,000 ng/mL) of THC-D_3 and THC-COOH-D_3. Label the flask "Cannabinoid Internal Standard". Include on the label initials, date prepared, and the expiration date which is determined from earliest date listed on the acquired drug standards.
Example: From separate drug standards containing 100 micrograms/mL of THC-D_3 and THC-COOH-D_3, transfer 1.0 mL from each standard to the same 100 mL volumetric flask. Dilute the flask to volume with methanol.

Calibration and Verification Solutions

a. Cannabinoid Calibrator Solution: from the individual drug standards prepare a solution that contains 1.0 microgram/mL (1,000 ng/mL) of THC and THC-COOH. Label this solution "Cannabinoid Calibrator Solution" and include initials, the

date prepared and the expiration date which is determined from the earliest date listed on the acquired drug standards.
Example: Dilute 1.0 mL of a 1.0 mg/mL THC standard to 10 mL with methanol. Place 1.0 mL of this solution in a 100 mL volumetric flask and add 1.0 mL of a 100 microgram/mL THC-COOH standard. Dilute the flask to volume with methanol.

b. Cannabinoid Verification Solution must be prepared using the same procedure to prepare the calibrator solution.

c. 50 ng/mL cannabinoid calibration/verification standard: Add 50 microliters of the cannabinoid calibration/verification solution to 1.0 mL of drug free blood. Add 100 microliters of the internal standard solution. Vortex the mixture.
d. 100 ng/mL cannabinoid calibration/verification standard: Add 100 microliters of the cannabinoid calibration/verification solution to 1.0 mL of drug free blood. Add 100 microliters of the internal standard solution. Vortex the mixture.
e. 200 ng/mL cannabinoid calibration/verification standard: Add 200 microliters of the cannabinoid calibration/verification standard to 1.0 mL of drug free blood. Add 100 microliters of the internal standard solution. Vortex the mixture.

Procedure:

1. Blood Sample Preparation:
   a. To 1 mL of blood add 1 mL of acetonitrile and 100 microliters of the internal standard.
   b. Mix/Vortex and let stand for 5 minutes.
   c. Sonicate sample for at least 1 minute. (This step is not required)
   d. Mix/Vortex samples.
   e. Centrifuge for 10 minutes at >2000 RPM
   f. Decant liquid portion of the sample into 5 mL of 0.1M Sodium Acetate buffer pH 4.5. Mix.

2. Extraction Procedure

   a. Condition column with 3 mL MeOH.
   b. Condition column with 3 mL water.
   c. Condition column with 1 mL 0.1M HCl.
   d. Load sample onto column.
   e. Rinse column with 2 mL water.
   f. Rinse column with 2 mL (0.1M HCL : Acetonitrile (70:30))

g. Dry column for 3 minutes with nitrogen
h. Rinse column with 0.2 mL Hexane.
i. Collect cannabinoids with 3 mL of (Hexane : Ethyl acetate (75:25))

Reagent flow must be between 1 to 15 mL per minute, except for elution reagent and samples which cannot be more than 5 mL per minute.

Post Extraction Procedure:

1. Evaporate the solvent from the collection test tube.

2. If desired, prior to derivatization the eluate may be transferred from the collection test tube to a vial using a hexane : ethyl acetate (75:25) mixture.

3. Derivatize by adding 50 microliters BSTFA with 1% TMCS to the vial or collection test tube and capping. Mix and heat the vial or collection test tube at 80^{o} C for 30 minutes. Remove from heat source and allow the vial or collection test tube to cool before analysis.

4. Examine the extract utilizing gas or liquid chromatography/mass spectrometry.

Quality Control:

Quality control is verified for each extraction by utilizing the internal standard. For each set of extractions a blood blank must be extracted as a negative control. At least one verifier must be extracted as a positive control. Reported quantitative values must be based on a calibration curve using at least three calibration standards, and at least one verification standard must be extracted to verify the calibration curve. The verification standard must quantify within +/- 20% of the target for quantitative values to be reported.

Personnel Safety Concerns:

When working with biohazardous samples use protective measures, such as gloves, eye protection, and work with the samples in a biosafety hood.

BSTFA with 1% TMCS should be handled in a fume hood, with gloves, and eye protection.

Maintenance:

Zymark: RapidTrace SPE Workstation

a. Check reagent levels daily before using.
b. Clean protein build-up when needed by passing about 7.5 mL 2N NaOH and 2N HNO_3 through the cannula to the aqueous waste.

Comments:

For an explanation of the operation of the RapidTrace refer to the operation manual.

Literature References:

RapidTrace SPE Workstation Installation and Quick Reference Manual, revision 0, Zymark Co., 1995.

Clean Screen® Extraction Column Applications Manual, United Chemical Technologies, Inc., Bristol, PA.