Name of Procedure:

Toxicology
THC and THC-COOH Extraction Procedure Using United Chemical Technologies Styre 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 Styre Screen Extraction Columns▪. This 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
Pipettes
Pipette tips
Volumetric flasks
World Wide Monitoring Styre Screen Extraction Columns▪
Zymark RapidTrace SPE Workstation or other SPE device
Zymark TurboVap LV or other evaporation device

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
Hexane
Ethyl Acetate
Acetonitrile

Concentrated Ammonium Hydroxide
Glacial Acetic Acid
Deionized Water **:** Acetonitrile **:** Concentrated Ammonium Hydroxide (84:15:1) mixture
(prepare reagent on the same day of extractions)
Hexane **:** Ethyl Acetate **:** Glacial Acetic Acid (49:49:2) mixture
(prepare reagent on the same day of extractions)
BSTFA with 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamine with 1% trimethylchlorosilane)

Internal Standard Solution

a. Internal Standard: Prepare an internal standard solution that contains 1.0 μg/mL (1,000 ng/mL) of THC-D_3 and THC-COOH-D_3. Label this solution ◘Cannabinoid Internal Standard◘ and 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 μg/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 μg/mL (1,000 ng/mL) of THC and THC-COOH. Label this solution ◘Cannabinoid Calibrator Solution◘ and include on the label 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 μg/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 μL of the cannabinoid calibration/verification solution to 1.0 mL of drug free blood. Add 100 μL of the internal standard solution. Vortex the mixture.
d. 100 ng/mL cannabinoid calibration/verification standard: Add 100 μL of the cannabinoid calibration/verification solution to 1.0 mL of drug free blood. Add 100 μL of the internal standard solution. Vortex the mixture.

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

Procedure:

1. Blood Sample Preparation:
   a. To 1 mL of blood slowly add 2 mL of cold acetonitrile and 100 μL of the internal standard.
   b. Mix/Vortex samples and let stand for 5 minutes.
   c. Mix/Vortex samples.
   e. Centrifuge for 10 minutes at >2000 RPM
   f. Decant liquid portion of the sample into a clean test tube and add 2.0 mL of deionized water.

2. Extraction Procedure

   a. Load sample onto column.
   b. Rinse column with 1.0 mL of the Water:Acetonitrile:Concentrated Ammonium Hydroxide reagent. The flow rate should be between 1 and 15 mL per minute.
   c. Dry column for 15 minutes.
   d. Collect cannabinoids with 3 mL of the Hexane:Ethyl Acetate:Glacial Acetic Acid reagent. The flow rate should not exceed 5 mL per minute.

Note: The method of extraction (RapidTrace SPE vs. vacuum box) is discretionary.

Post Extraction Procedure:

1. Evaporate the solvent from the collection test tube.

2. Derivatize by adding 50 μL BSTFA with 1% TMCS to the vial or collection test tube and capping. Mix and heat the vial or collection test tube at 80° C for 30 minutes. Remove from heat source and allow the vial or collection test tube to cool before analysis.

3. 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.

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.

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.

Styre Screen◘ Extraction Column Applications Manual, United Chemical Technologies, Inc., Bristol, PA.