



Solid phase extraction of THC, THC-COOH and 11-OH-THC from whole blood

Application Note

Forensic Toxicology

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Introduction

Marijuana is derived from *Cannabis Sativa*. There are more than 400 chemicals in the cannabis plant. The Δ^9 -tetrahydrocannabinol (THC) is the most psychoactive of the various forms of THC. Marijuana is most often used in cigarette form, the user inhaling the marijuana smoke. THC and other forms of cannabinoids are lipid soluble and can enter body tissues rapidly. THC is rapidly metabolized to 11-hydroxy- Δ^9 -tetrahydrocannabinol (11-OH-THC), which is then converted to 11-nor- Δ^9 -THC-9-carboxylic acid (THC-COOH).

Detection of THC metabolites in urine, primarily THC-COOH, can indicate prior THC exposure but provides no indication of impairment. Testing for THC and its metabolites in blood can give a better indication of recent drug usage and can be of merit when testing for impairment.

This application note shows an effective SPE method for the extraction of THC and key metabolites from human blood and the GC/MS analysis of these compounds.



Instrumentation

GC with single quad mass spectrometer

Materials and Reagents

High flow Agilent Bond Elut Certify II SPE cartridge 200 mg (p/n 14113051). Bond Elut Certify II is a mix of C8 and a quaternary amine, a strong anion-exchange bonded silica. The two functionalities are effective in retaining the polar and non - polar functionalities of the THC compounds

5% phenyl substituted, low bleed GC/MS column 30 m x 0.25 mm x 0.25 µm

d3-THC, d3-11-OH-THC and d9-carboxy-THC from Cerilliant

Sample Preparation

Pipette 2 mL blood into a clean tube with ISTD equivalent to 10 - 11g/L (ng/mL)

Add 4 mL cold acetonitrile drop-wise while vortexing

Centrifuge sample 5 min minimum 2500 rpm

Transfer supernatant to a clean labeled tube.

Evaporate sample to about 3 mL with nitrogen at 35 - 40 °C

Add 7 mL 0.1 M sodium acetate buffer, pH 6.0 to each sample

SPE Method

Conditioning

Condition Certify cartridge with 2 mL MeOH. (All steps, except where noted, utilize low vacuum of approximately 2 - 5 in Hg).

Condition cartridge next with 2 mL 0.1 M sodium acetate buffer, pH 6.0 with 5% MeOH.

Cartridges should not be allowed to dry prior to sample addition.

Pour sample into column reservoir and draw sample through the column slowly, 1-2 mL/min.

Washes

2 mL sodium acetate buffer, pH 6.0.

Dry column under maximum vacuum for approximately 5 minutes.

Wash with 1 mL hexane

Elution

Elute THC with 2 mL 95:5 hexane:ethyl acetate.

Wash column with 5 mL 1:1 MeOH:DI water.

Dry column under maximum vacuum for approximately 5 minutes.

Wash with 1 mL hexanes.

Elute (in a separate tube) THC-COOH and 11-OH-THC with 2 mL 1% acetic acid in 75:25 hexane:ethyl acetate.

For best results, do not combine fractions. Run as two samples. Evaporate elution fractions under nitrogen no higher than 40 °C.

Derivatization

Add 500 µL elution solvent to sample, vortex and transfer to a clean, high recovery GC vial. Evaporate to dryness with nitrogen no higher than 40 °C

Add 35 µL BSTFA with 1% TMCS and 35 µL ethyl acetate. Overlay samples with nitrogen, cap and heat 20 minutes at 70 °C

Conditions

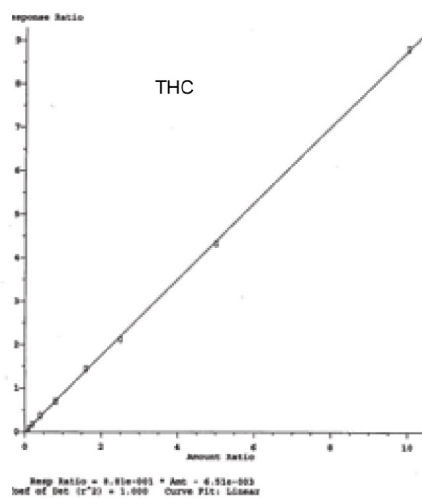
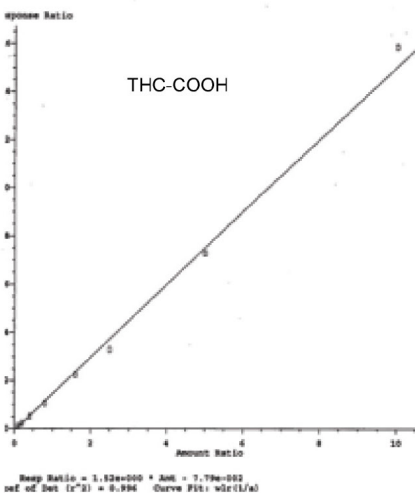
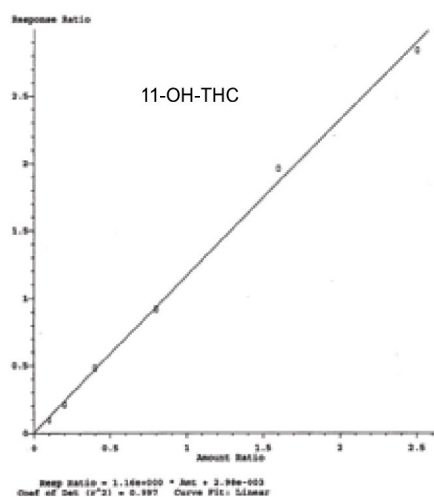
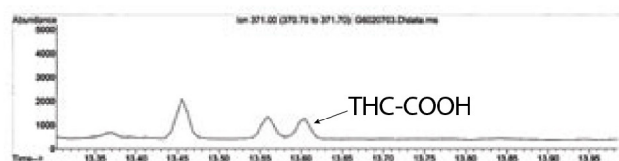
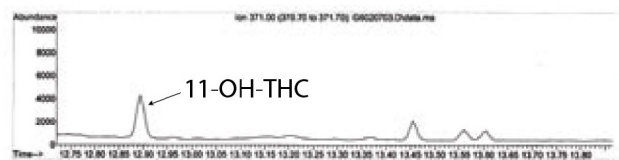
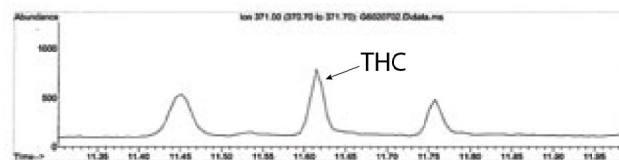
Inlet temperature:	250 °C
Mode:	Pulsed pressure injection
Injection volume:	2 µL
Initial oven temperature:	120 °C Hold 1 min 15 °C/min to 300 °C Hold 0 30 °C/min to 310 °C Hold 5.57 min

Target Ions

d3-THC 374, 389, 346 (dwell time 50 ms)
 THC 371, 386, 343
 Linear range 1 - 50 µg/L

d3-11-OH-THC 374, 462, 477
 11-OH-THC 371, 459, 474
 Linear Range 1 - 50 µg/L

d9-THC-COOH 380, 479, 497
 THC-COOH 371, 473, 488
 Linear Range 1 - 100 µg/L



Conclusion

The above data shows the effective use of mixed-mode SPE with GC/MS detection for the extraction and quantification of THC and key metabolites from whole blood at low levels.

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