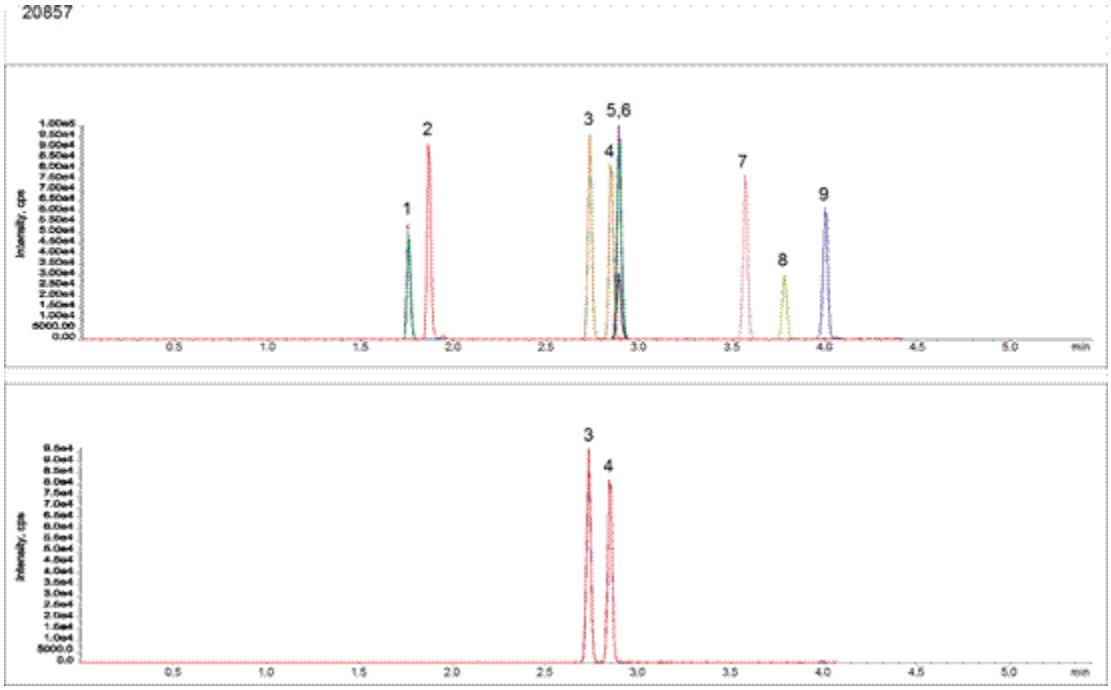
Analysis of Cannabinoids and Other Cannabis Constituents such as Δ9-tetrahydrocannabinol (THC) and its Metabolites by LC-MS-MS and GC-MS

Method 1: Cannabinoid Metabolites by LC-MS-MS

Instrumentation: Waters ACQUITY UPLC system **with** AB Sciex LC/MS/MS System
Column: Waters XBridge C18 2.5µm 2.1 X 50mm Column (Ctrl + Click to follow link)
Elution Type: Gradient
Elution A: 10mM Ammonium Formate
Elution B: Acetonitrile
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	80	20
2	4	0	100

Flow Rate: 0.5 mL/min
Col. Temp: Ambient
Detection: Tandem Mass Spec (MS-MS) **@ amu (22°C)** (Ctrl + Click to follow link)
Detector Info: AB Sciex API 4000 (Ctrl + Click to follow link)
Typical MS Conditions
Source: Positive ESI
Vaporizer: 330 °C
Capillary temp: 270 °C
Sheath Gas (AU): 35
Aux Gas (AU): 25
In Sweep Gas (AU): 5
Collision Gas Pressure: 25 torr

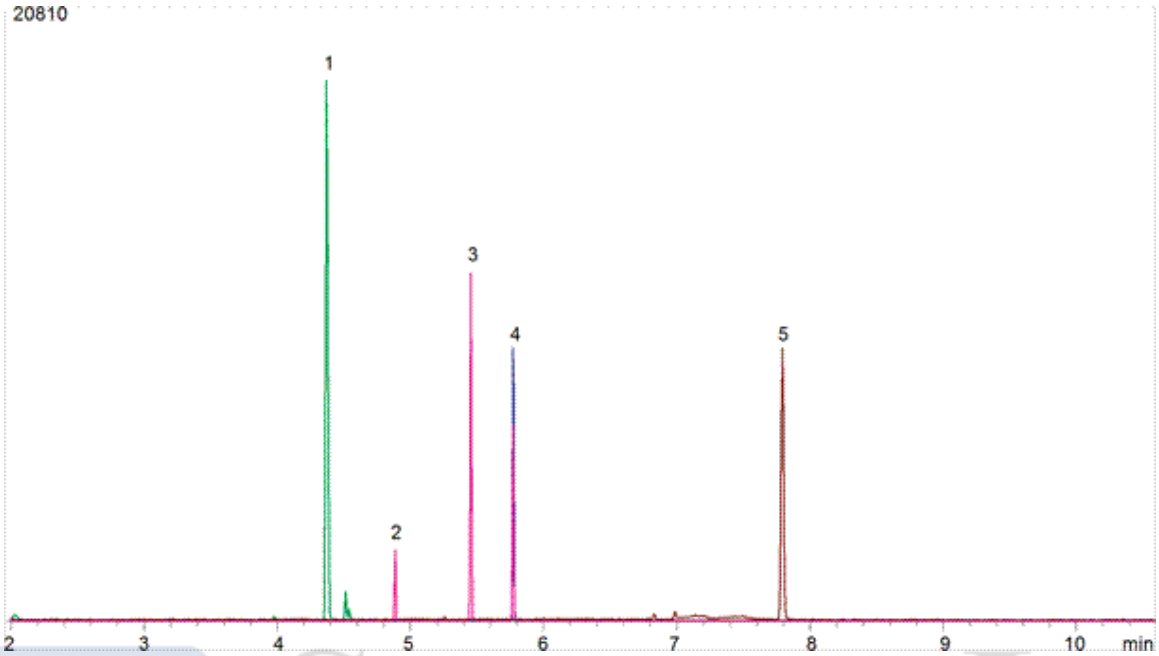


1: JWH-073-Butanoic acid metabolite
2: JWH-018-Pentanoic acid metabolite
3: JWH-073-4-Hydroxybutyl metabolite
4: JWH-073-3-Hydroxybutyl metabolite
5: AM2201-4-Hydroxypentyl metabolite
6: JWH-018-4-Hydroxypentyl metabolite
7: AM2201
8: JWH073
9: JWH018

Method 2: Cannabinoids and Synthetic Opiates by GC-MS

Instrumentation: GC/MS with Split/Splitless Injector (Ctrl + Click to follow link)
Column: Zebron™ ZB-5MS, GC Cap. Column 20 m x 0.18 mm x 0.18 µm
Injection Mode: Split/Splitless
Injection Type: Splitless
Carrier Gases: Helium
Col./Oven Temp: 150 °C hold 1 min, to 320 °C at 35 °C/min, then hold 5 min
Inlet temperature: 250 °C
Injection volume: 1 µL
Detection: MSD @ 320 °C (Ctrl + Click to follow link)
Sim mode: Methadone (294), THC (371), THC-OH (371), THC-COOH (473), Buprenorphine (450)

Sample Preparation: Sample is derivatized with BSTFA at 70ºC for 30 minutes.



1: Methadone
2: THC
3: THC-OH
4: THC-COOH
5: Buprenorphine

Method 3: Cannabinoids and Synthetic Opiates by GC-MS

Instrumentation: GC/MS with Programmable Split/Splitless EPC Injector (Ctrl + Click to follow link)
Pre-Column: 1 m section from a 15 m × 0.25 mm, 0.25 µm HP-5 ms Ultra Inert column
Analytical Column: 15 m × 0.25 mm, 0.25 µm DB-17 ms
Injection Mode: Programmable Split/Splitless
Injection Type: Splitless
Carrier Gases: Helium in constant pressure mode.
Pressures: Pre-column: 1 psi; Column 1: 5 psi; Column 2: 9.6 psi
Col./Oven Temp: 100 °C hold 50 seconds, to 230 °C at 200 °C/min, from 230 °C to 280 °C at 10 °C/min then hold at 280 °C for 1 min
Inlet temperature: 280 °C
Injection volume: 1 µL
Transfer Line Temp: 300 °C
Detection: MSD (Ctrl + Click to follow link)
Ion Source temperature: 230 °C
Source: Positive ESI
Collision Gas: Nitrogen constant flow 1.5 mL/min
Quench gas: Helium constant flow 1.5 mL/min
Solvent delay: 3.0 min

Sample Preparation:

A 2 mL blood sample containing 10 µg/mL of each internal standard (ISTD) and spiked with THC, 11-OH-THC and THCA was pipetted into a clean tube, and 4 mL of acetonitrile was added. After centrifugation at 2500 rpm for 5 minutes, the supernatant was transferred and evaporated to about 3 mL with nitrogen at 35-40 °C, and 7 mL of 0.1 M sodium acetate (pH 6.0) was added.

High Flow Bond Elut Certify II SPE columns were conditioned with 2 mL of methanol, then 2 mL 0.1 M sodium acetate buffer, pH 6.0 with 5% methanol. Cartridges were not be allowed to go to dryness prior to sample addition. The sample was drawn through the column slowly, at 1 to 2 mL/min. The column was then washed 2 mL sodium acetate buffer, pH 6.0, dried under maximum vacuum for approximately 5 minutes, then washed with 1 mL hexanes. THC was eluted under neutral conditions with 2 mL of 95:5 hexane: ethyl acetate. This was followed by a 5 mL 1:1 methanol:deionized water wash. The column was again dried under maximum vacuum for approximately 5 minutes and washed again with 1 mL hexanes. Elution of 11-OH-THC and THCA was performed with 2 mL 1% acetic acid in 75:25 hexane:ethyl acetate. The THC and the metabolite fractions were combined and dried before derivatization.

The eluent was evaporated under nitrogen at a temperature no higher than 40 °C, then reconstituted in 60 µL of toluene and 40 µL of BSTFA, 1% TMCS for derivatization. The sample tubes were capped and heated 20 minutes at 70 °C before injection.

Results:

This method has a dynamic range of 0.1 to 50 ng/mL for THC and 11-OH-THC, and 1 to 100 ng/mL for THCA which match industry norms. The accuracy of quantification is also quite good, with an R2 of 0.999 for all three analytes THC (D9-Tetrahydrocannabinol), 11-OH-THC (11-hydoxy-D9-tetrahydrocannabinol), and THCA (11-nor-D9-Tetrahydrocannabinol-9-Carboxylic Acid).

References:

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