Optimized SPE for UPLC-MS/MS and GC-MS/MS Determination of THC and Metabolites in Urine and Blood

Michael S. Young and John T. Martin
Waters Corporation, 34 Maple street, Milford, MA

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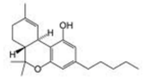
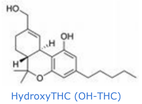
INTRODUCTION

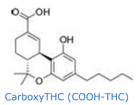
Δ9-Tetrahydrocannabinol (THC) is the principal psychoactive constituent of the cannabis products marijuana and hashish. After smoking or oral ingestion of cannabis, THC is incorporated into the bloodstream and is available for transport to receptor sites and for metabolism. Among the important THC metabolites are hydroxy-THC (OH-THC), which is also psychoactive, and carboxy-THC (COOH-THC) which is not psychoactive but may have analgesic properties (see structures below). THC and its metabolites may be detected in the blood and urine of users. In blood, THC is detectable for many hours after ingestion and is indicative of recent cannabis ingestion. Therefore, such analysis is evidence that the user may have been under the influence of THC at the time the sample was collected. The THC level in the urine of users is generally very low; the principal analyte in urine is the COOH-THC metabolite. COOH-THC may be detectable in urine samples many hours or even days after ingestion of cannabis. Although the presence of the non-psychoactive metabolite is evidence that the subject has recently used cannabis, it is not necessarily evidence that the user was under the influence of THC at the time the sample was collected.



Δ9-Tetrahydrocannabinol (THC)



HydroxyTHC (OH-THC)



CarboxyTHC (COOH-THC)

The goals of this work:

- To develop a single SPE based analytical protocol suitable for determination of THC, OH-THC and COOH-THC in either blood or urine samples and appropriate for either GC or LC based analysis
- To develop a tandem UPLC-MS method for THC and metabolites
- To develop a tandem GC-MS method for THC and metabolites

SPE PROTOCOL

Sample Pre-Preparation

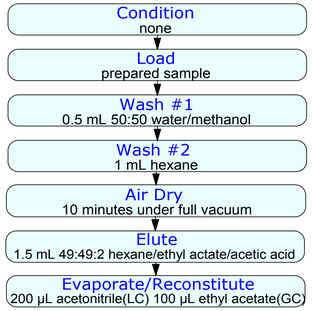
Blood

Samples (0.5 mL) are precipitated by dropwise addition of 1 mL acetonitrile while vortex mixing. After centrifugation, 1 mL of the supernatant is diluted to 2.5 mL with 1 % aqueous ammonia. The resulting solution is loaded onto the SPE cartridge.

Urine

Samples (2 mL) are hydrolyzed by addition of 50 µL of 10 N NaOH solution followed by heating at 60°C for 15 minutes. After cooling, the samples are adjusted to pH 7 by addition of 50 µL of 50% aqueous acetic acid and 200 µL of 0.1 M pH 7 phosphate buffer. 1 mL of acetonitrile is added to the prepared sample and the resulting solution is loaded onto the SPE cartridge.

Solid-Phase Extraction (Oasis MAX, 3 cc Cartridge)



Recovery is 85-93 % (< 10% RSD) and ion-suppression is under 10 % for all analytes in urine or precipitated blood.

Cleanup is accomplished using solvents chosen to elute the analytes but leave polar interferences on cartridge.

Sample Analysis

LC-MS

Add 150 µL water, mix well and analyze by UPLC-MS/MS.

GC-MS

The sample is eluted, evaporated and reconstituted in a suitable vial with Teflon lined cap. Add 50 µL of derivatization reagent (BSTFA/TMCS 99:1) and place the tightly capped vial into a 70°C oven for 15 minutes. After cooling, the derivatized sample is analyzed by GC-MS/MS.

UPLC®-MS/MS

Acquity UPLC

Column: Acquity BEH C18 (2.1 x100)
Mobile Phase: A) 0.1% formic acid/water
B) acetonitrile
Flow: 400 µL/min
Gradient: linear, 60% B to 90% B in 4 min
hold at 90% B to 4.3 min
to 60% B at 4.5 min
Injection: 10 µL (full loop)
Temp: 30°C

Quattro Premier XE Mass-Spectrometer

MRM Transitions	Cone(V)	Collision(eV)
THC (ES+)		
315>193	40	25
315>259	40	25
318>196 (d3-ISTD)	40	25
OH-THC (ES+)		
331>201	35	24
331>313	35	15
334>316 (d3-ISTD)	35	15
COOH-THC (ES-)		
343>245	40	30
343>299	40	25
346>302 (d3-ISTD)	40	25

GC-MS/MS

A6890 GC

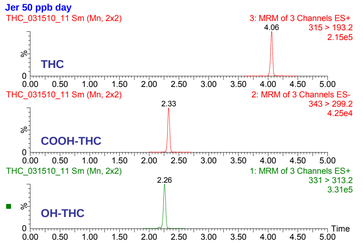
Column: RTX-5MS (30m x 0.025mm x 0.25µm *df*)
Carrier Gas: Helium
Flow: 1.0 mL/min (constant flow)
Temperature Program:
100°C for 1.5 min
40°C/min to 200°C, hold 4 min
8°C/min to 240°C, hold 1 min

Quattro micro GC Mass-Spectrometer

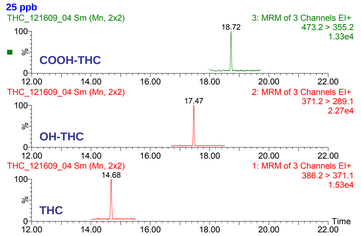
MRM Transitions	Collision(eV)
THC	
371>289	10
386>371	10
389>374 (d3-ISTD)	10
OH-THC	
371>265	10
371>289	15
374>292 (d3-ISTD)	15
COOH-THC	
371>289	12
473>355	20
374>292 (d3-ISTD)	12

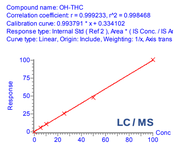
CHROMATOGRAPHY

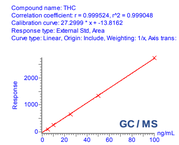
Typical LC/MS Chromatogram
50 ng/L in Urine



Typical GC/MS Chromatogram
25 ng/L in Blood





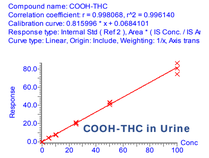


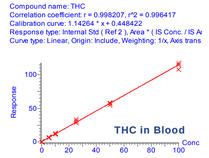
- For both GC-MS and LC-MS, linear response was observed from 0 to 100 ng/L
  - LC-MS analysis in five minutes, GC-MS analysis in **25 minutes**
- The best LC-MS signal for OH-THC was loss of H2O and for COOH-THC was loss of COOH
  - MRM transitions based on loss of water or carboxy may be prone to interference
  - no interference was seen for two types of blood and four types of urine

RESULTS

Intraday

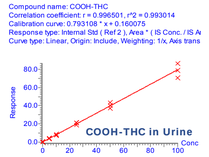
Three blood and urine calibration curves were prepared on a single day to measure the intraday reproducibility of the SPE and analysis methods. The results are summarized below (total of 18 measurements for each evaluation). Similar results were seen for the other analytes in urine and blood.

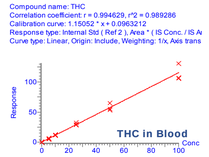




Interday

Three blood and urine calibration curves were prepared on three succesive days to measure the interday reproducibility of the SPE and analysis methods. The results are summarized below (total of 18 measurements for each evaluation). Similar results were seen for the other analytes in urine and blood.





CONCLUSIONS

- Results show that the performance of the Oasis MAX SPE protocol is equal or superior to competitor silica based THC cartridge
- Interday and intraday method performance is sensitive and reproducible
  - LOQ below 5 ng/L
  - Intraday reproducibility of internal standard area was better than 10 % RSD for all analytes in blood and urine
- The same SPE protocol may be used for GC-MS or LC-MS samples
  - UPLC-MS method is faster and more straightforward
    - no derivatization
    - faster chromatography
  - GC-MS evaluation for urine will be completed in time for presentation at ACS fall meeting (ORAL) and SOFT meeting (POSTER).

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