A Comparative Study for Method Development and Validation for the Detection of THC, THC-OH, and THC-COOH in Blood using GC/MS and LC/MS/MS

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Introduction

Cannabis sativa, or marijuana, is a plant that contains over 70 psychoactive compounds, known as cannabinoids. The primary psychoactive cannabinoid in marijuana is Δ-9-tetrahydrocannabinol (THC). THC is converted to THC-OH and THC-COOH during metabolism (Figure 1). THC and THC-OH are psychoactive and THC-COOH is non-psychoactive. Cannabinoids bind to and stimulate the CB1 and CB2 receptors that are located in the brain, spinal cord, and other peripheral locations in the body.

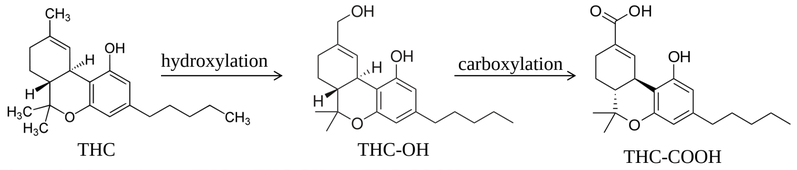

Figure 1: Metabolism of THC to THC-OH and THC-COOH

Federally, marijuana is classified as a Schedule 1 drug, meaning it has a high potential for abuse and no accepted medical use. Recently, marijuana has been legalized for medicinal use and/or recreational use at the state level. Since marijuana causes impairment, a level of THC and metabolites in the blood needs to be determined for the indication of intoxication while driving, similar to alcohol. With increased use and legalization, it is important to have methods in place for the detection of THC and metabolites to properly quantify whether an individual is impaired. With per se limits set to 1 ng/mL for THC and 5 ng/mL for THC-OH and THC-COOH in many states, development of sensitive, selective, and reliable methods of detection are increasingly more important. This project aims to represent the results found in comparison of the analytical platforms GC/MS and LC/MS/MS for the quantification of THC and metabolites. GC/MS has commonly been used for the detection of THC and metabolites in blood, while LC/MS/MS offers a newer approach to increase specificity with the use of tandem mass spectrometry and to decrease sample preparation.

Methods

Liquid-Liquid Extraction for Analysis by LC/MS/MS

1. 1 mL of blood sample was aliquoted
2. Internal standard was added to all samples
   - Internal Standard: 100 ng/mL (THC-D3, THC-OH-D3, THC-COOH-D3)
3. Samples were vortexed to homogeneity
4. 2.5 mL of cold acetonitrile was added while vortexing to protein crash
5. Samples were centrifuged, acetonitrile layer was transferred and dried down to ~0.5 mL
6. 1 mL of 5% phosphoric acid in water was added and samples were vortexed
7. 3 mL of elution solvent (80/10/10: hexanes/ethyl acetate/MTBE) was added
8. Samples were capped, rotated for 15 minutes, and centrifuged
9. Supernatant was transferred using the freeze/pour acetone bath method
10. Samples were dried down at 55ºC for 20 minutes
11. Samples were reconstituted in 50 µL of 50:50 mobile phase
12. Samples were analyzed by ESI$^+$ LC/MS/MS (Acquity UPLC© BEH C18 column)
    - Mobile Phase A: 0.1% formic acid in water
    - Mobile Phase B: 0.1% formic acid in acetonitrile

Table 1: ESI$^+$ MS/MS Conditions

Analyte	Cone (V)	Collision (eV)	MRM Transitions
THC	35	20	315.2>135.1 315.2>193.2
THC-OH	30	25	331.2>201.2 331.2>313.2
THC-COOH	35	20	345.2>299.2 345.2>327.2

Results

Gas Chromatography/Mass Spectrometry

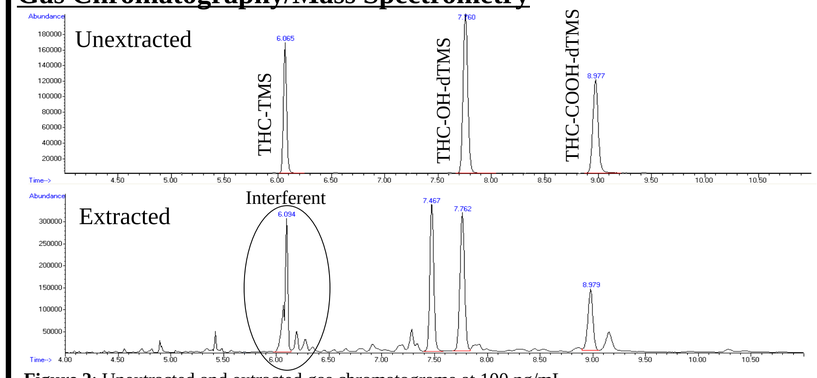

Figure 2: Unextracted and extracted gas chromatograms at 100 ng/mL

Liquid Chromatography/Tandem Mass Spectrometry

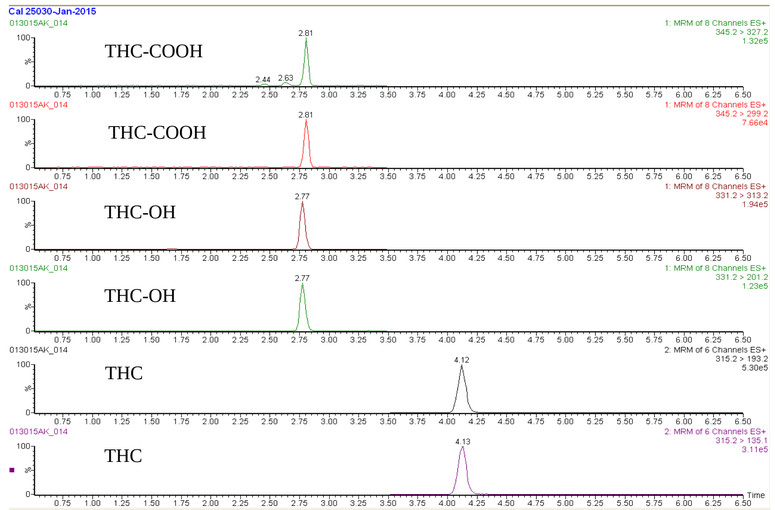

Figure 3: Chromatogram of 250 ng/mL calibrator

Method Validation using LC/MS/MS

- This method was validated following the SWGTOX Standard Practices for Method Validation in Forensic Toxicology Guidelines
- Percent recoveries were as follows:
  - THC – 70%, THC-OH – 65% and THC-COOH – 59%

Table 2: Linear Dynamic Range for Calibration Models

Analyte	Range (ng/mL)	Controls (ng/mL)	Correlation Coefficient (R^2)
THC	1 - 250	Low : 3 High: 200	0.996
THC-OH	5 - 250	Low : 5 High: 200	0.997
THC-COOH	5 - 250	Low : 5 High: 200	0.996

Acceptability Criteria: correlation coefficient $R^2 \geq 0.98$

Results

Table 3: Accuracy (Bias %)

Analyte	LLOQ Control	Low Control	High Control
THC	-13.73	-19.08	-14.64
THC-OH	-6.12	-9.71	-15.37
THC-COOH	-18.91*	-18.87*	-4.38

Acceptability Criteria: maximum bias ±20%

Table 4: Within and Between Run Precision (Coefficient of Variation %)

Analyte	LLOQ Control	Low Control	High Control
THC	11.63	9.91	6.95
THC-OH	18.22	18.51	10.36
THC-COOH	16.24*	16.50*	7.12

Acceptability Criteria: maximum variation ±20%

Table 5: Dilution Integrity

Analyte	1:2 (50 ng/mL)		1:10 (50 ng/mL)	
	Bias (%)	CV (%)	Bias %	CV (%)
THC	-3.80	11.21	-14.50	7.42
THC-OH	-11.98	17.05	-19.70	16.55
THC-COOH	-7.71	10.00	-12.19	13.45

Acceptability Criteria: maximum variation and bias ±20%

*denotes incomplete sample set

Conclusions

- GC/MS was determined to not be a suitable means of analysis for blood containing THC, THC-OH, and THC-COOH in our laboratory. It was determined that interferences with endogenous compounds in blood (Figure 2) were unable to be chromatographically resolved and therefore the quantitation of THC was inhibited.
- LC/MS/MS was determined to be the preferred means of analysis for blood containing THC, THC-OH, and THC-COOH. The developed method was validated using the SWGTOX guidelines with all results within acceptable ranges.
- Linear calibration model: all correlation coefficients at or above 0.996 (Table 2).
- Accuracy and precision: all bias percentages within 20% (Table 3) and all variation, between and within run, below 20% (Table 4).
- Dilution integrity: both bias and variation within and below 20% (Table 5).
- Recovery: below generally accepted percentage of 70%, but there is no acceptability criteria associated with recovery according to SWGTOX guidelines

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