

City University of New York (CUNY)

CUNY Academic Works

Student Theses

John Jay College of Criminal Justice

Spring 5-2020

Analysis of Cannabinoids in Serum by GC-MS/MS

Christie Cannarozzi

CUNY John Jay College, christie.cannarozzi@jjay.cuny.edu

[How does access to this work benefit you? Let us know!](#)

More information about this work at: https://academicworks.cuny.edu/jj_etds/147

Discover additional works at: <https://academicworks.cuny.edu>

This work is made publicly available by the City University of New York (CUNY).

Contact: AcademicWorks@cuny.edu

Analysis of Cannabinoids in Serum by GC-MS/MS

A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Forensic Science
John Jay College of Criminal Justice
The City University of New York

Christie Cannarozzi
May 2020

Analysis of Cannabinoids in Serum by GC-MS/MS

Christie Cannarozzi

This Thesis Has Been Presented to and Accepted by the Office of Graduate Studies, John Jay College of Criminal Justice in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science

Thesis Committee:

Thesis Advisor: Dr. Marta Concheiro-Guisan

Second Reader: Dr. Gail Cooper

Third Reader: Dr. Garry Milman

Table of Contents	Pages
Acknowledgments	i
Abstract	ii
List of Tables	iii – iv
List of Figures	v
Introduction	1 – 4
Materials and Methods	4 – 13
1. Reagents and Supplies	4 – 5
2. Instrumental Parameters	5 – 7
3. Preparation of the Calibrator and QC Samples	8 – 9
4. Sample Extraction	9 – 10
5. Method Cross-Validation	10 – 12
6. Identification and Quantification Criteria	12 – 13
7. Authentic Case Sample Analysis	13
Results	13 – 24
1. GC-MS/MS Method Cross-Validation in Serum	13 – 18
2. Comparison of Validation Parameters in Serum vs. Blood	18 – 21
3. Authentic Case Sample Analysis	22 – 24
Discussion	25 – 26
Conclusion	27
References	28 – 31
Appendix	32 – 37

Acknowledgements

I would like to express my sincerest gratitude to Dr. Marta Concheiro-Guisan, who served as my thesis mentor for the past year. I wouldn't be where I am today if it were not for her guidance, patience, and instruction in forensic toxicology. I would also like to sincerely thank Dr. Gail Cooper for providing me with an interesting thesis topic and the opportunity to perform my thesis research at the New York City Office of the Chief Medical Examiner (NYC-OCME). Next, I would like to acknowledge the criminalists at the NYC-OCME, specifically Justine Pardi for always being so helpful and answering my questions regarding method validation and GC-MS/MS. I would also like to acknowledge Dr. Garry Milman for agreeing to be my third reader. Lastly, I would like to thank my family, boyfriend, and classmates for supporting me throughout the past two years in graduate school. This accomplishment would not have been possible if it were not for the continuous encouragement from all these individuals.

Abstract

Due to recent changes in federal and state legislations, the availability and consumption of cannabis products have increased in the United States. The expanded use of recreational and medicinal cannabis products increases the importance of implementing sensitive and selective instrumental methods in toxicological laboratories, as legal implications may arise in forensic cases, such as driving under the influence of drugs (DUID). The purpose of this study was to perform a cross-validation for the quantitative analysis of cannabinoids (Δ^9 -tetrahydrocannabinol, cannabidiol, cannabinol, 11-hydroxy- Δ^9 -tetrahydrocannabinol, and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol) in serum by gas chromatography coupled to triple quadrupole tandem mass spectrometry (GCTQ). This method was fully validated following the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. Linearity was established within 1-100 ng/ml; bias was within $\pm 20\%$ and imprecision was less than 20%; limits of detection (LOD) and quantitation (LOQ) were 1 ng/mL; and extraction efficiency ranged from 51.3 to 58.2%. Furthermore, the present method and a previously developed method for the determination of cannabinoids in blood were applied to serum (n = 20) and blood (n = 16) authentic case samples obtained from the New York City Office of the Chief Medical Examiner (NYC-OCME) to investigate a correlation between cannabinoid concentration in serum versus blood.

List of Tables	Page
Table 1. MRM GC-MS/MS parameters for the derivatized target analytes and internal standards in serum (n = 8).	7
Table 2. Guidelines for the preparation of the calibrator and QC samples in 0.5 mL of biological sample (blood or serum).	8
Table 3. Summary of bias results at each QC concentration (3, 20, and 70 ng/mL) for the target analytes in serum (n = 15).	15
Table 4. Summary of imprecision results at each QC concentration (3, 20, and 70 ng/mL) for the target analytes in serum (n = 15).	15
Table 5. Summary of bias and imprecision results at the LOD and LOQ (1 ng/mL) for the target analytes in serum (n = 9).	16
Table 6. Summary of extraction efficiency results at the low (3 ng/mL) and high (70 ng/mL) QC concentrations for the target analytes in serum (n = 5).	17
Table 7. Summary of dilution integrity results for the target analytes in serum (n = 3).	18

	Page
Table 8. Linearity parameters for the target analytes in blood (n = 5).	19
Table 9. Comparison of extraction efficiency results at the low (3 ng/mL) and high (70 ng/mL) QC concentrations for the target analytes in blood and serum (n = 5).	21
Table 10. Summary of target analyte concentrations in blood (n = 16) and serum (n = 20) authentic case samples.	23
Table 11. Ratio of THC-COOH concentration in blood to serum from paired blood and serum authentic cases (n = 8).	24

List of Figures	Page
Figure 1. Total ion chromatogram (TIC) of a low QC (3 ng/mL) sample in sheep serum.	14
Figure 2. Total ion chromatogram (TIC) of serum sample from case number 7.	22
Figure 3. Total ion chromatogram (TIC) of hospital blood sample from case number 7.	22
Figure 4. Scatter plot of THC-COOH concentration in serum versus blood from paired blood and serum authentic cases (n = 8).	24

Introduction

Cannabis, also known as marijuana, is a psychoactive substance that is obtained from the *Cannabis sativa* plant (Levine, 2003; Negrusz & Cooper, 2013). Its main psychoactive component is Δ^9 -tetrahydrocannabinol (THC); however, the plant contains over 140 pharmacologically active cannabinoids, such as cannabidiol (CBD) and cannabinol (CBN) (Levinsohn & Hill, 2020). In the human body, THC metabolizes into 11-hydroxy- Δ^9 -tetrahydrocannabinol (11-OH-THC), which is the primary active metabolite, and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH), which is the primary inactive metabolite (Levine, 2003).

Cannabis is the most commonly used illicit drug in the United States, with an estimated 43.5 million Americans aged 12 or older having reported using marijuana in 2018 (Substance Abuse and Mental Health Services Administration, 2019). However, cannabis also has been increasingly used therapeutically; it is estimated that more than two million Americans utilize cannabis for therapeutic purposes (Levinsohn & Hill, 2020). In regard to its clinical application, cannabis has been used to treat a variety of conditions, including multiple sclerosis, neuropathic pain, anxiety disorders, sleep disorders, appetite stimulation for HIV/AIDS patients, and nausea and vomiting due to chemotherapy (Citti, Braghiroli, Vandelli, & Cannazza, 2018). For example, Marinol® (dronabinol, a synthetic form of THC) and Cesamet® (nabilone, a derivative of THC) are medications that have been approved by the Food and Drug Administration (FDA) for the treatment of nausea and vomiting due to chemotherapy (Levinsohn & Hill, 2020). In addition, Epidiolex® is a medication containing CBD that has been approved by the FDA

for the treatment of drug-resistant seizures that result from Dravet syndrome or Lennox-Gastaut syndrome (Kicman & King, 2014; Marcoux, Holmes, & Vogenberg, 2019).

Currently, thirty-three states and the District of Columbia have enacted laws permitting the use of cannabis for medicinal purposes (Marcoux, Holmes, & Vogenberg, 2019; Peterman, 2019). Of these thirty-three states, eleven states and the District of Columbia have legalized the use of cannabis for recreational purposes too (Levinsohn & Hill, 2020). Despite its medicalization and legalization in several states, cannabis remains classified as a Schedule I drug under the Controlled Substances Act because of its high potential for abuse and absence of a currently accepted medical use in the US. Nonetheless, the 2018 Farm Bill legalized the production of hemp containing less than 0.3% of THC (Marcoux, Holmes, & Vogenberg, 2019).

Cannabis is the most commonly detected illicit drug in driving under the influence of drugs (DUID) cases (Compton, 2017; Hartman, Richman, Hayes, & Huestis, 2016). Studies have concluded that cannabis impairs one's driving ability due to its undesirable effects on reaction time, cognitive performance, divided attention, perception, and temporal and spatial orientation (Compton, 2017; Negrusz & Cooper, 2013). Specifically, slight driving impairment has been observed at THC serum concentrations between 2-5 ng/mL, whereas significant driving impairment has been observed at THC serum concentrations between 5-10 ng/mL (Urfer, Morton, Beall, Feldmann, & Gunesch, 2014). Furthermore, cannabis use is associated with a significant dose-related decrease in driving performance and an increased number of traffic crashes (Hartman et al., 2016; Kicman & King, 2014; Negrusz & Cooper, 2013).

All states have enacted laws that prohibit individuals from driving while impaired by alcohol and/or other drugs (Compton, 2017). As of May 2019, eighteen states have enacted *per se* laws that prohibit an individual from driving with a specified amount of THC, which is commonly 5 ng/mL but ranges from 1-10 ng/mL in blood (Hartman et al., 2016; Peterman, 2019). Other states have enacted zero tolerance laws that prohibit an individual from driving with any measurable amount of THC or its metabolites in the body (Compton, 2017). However, some studies have concluded that the concentration of THC and its metabolites in blood may not be an accurate and reliable predictor of the degree of impairment in an individual (Compton, 2017; Negrusz & Cooper, 2013; Peterman, 2019).

The expanded use of recreational and medicinal cannabis products increases the importance of implementing sensitive and selective instrumental methods in toxicological laboratories, as legal implications may arise in DUID cases and other forensic cases. Although blood tests are often performed for the determination of cannabinoids in DUID cases, other biological matrices that are commonly examined include serum and plasma (Citti et al., 2018; Compton, 2017; Peterman, 2019). Nonetheless, equivalence among cannabinoid concentrations in blood, serum, and plasma has been scarcely explored (Giroud et al., 2001; Raikos et al., 2014; Schaefer et al., 2015; Schwilke et al., 2009).

Solid phase extraction (SPE) or liquid-liquid extraction (LLE) followed by gas chromatography (GC) coupled to different types of detectors, such as the flame ionization detector (FID) and mass spectrometer (MS), has been traditionally employed for the simultaneous determination of cannabinoids in biological specimens (Gasse, Pfeiffer, Köhler, & Schürenkamp, 2016; Nahar, Guo, & Sarker, 2019; Purschke, Heinel, Lerch,

Erdmann, & Veit, 2016; Schwilke et al., 2009). In addition, gas chromatography tandem mass spectrometry (GC-MS/MS) methods using electron impact or chemical ionization have been implemented to increase the sensitivity and specificity of the assay (Andrenyak, Moody, Slawson, O’Leary, & Haney, 2017; Castro, Tarelho, Melo, & Franco, 2018; Nahar et al., 2019). More recently, liquid chromatography mass spectrometry (LC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods using electrospray ionization have been reported because they do not require derivatization (Citti et al., 2018; Gottardo, Sorio, Ballotari, & Tagliaro, 2019; Lacroix & Sausseureau, 2012; Raikos et al., 2014; Schaefer et al., 2015; Schwoppe, Scheidweiler, & Huestis, 2011).

The purpose of this study was to perform a cross-validation for the quantitative analysis of cannabinoids in serum by gas chromatography coupled to triple quadrupole tandem mass spectrometry (GCTQ). Furthermore, the present method and a previously developed method for the determination of cannabinoids in blood were applied to serum (n = 20) and blood (n = 16) authentic case samples obtained from the New York City Office of the Chief Medical Examiner (NYC-OCME) to investigate a correlation between cannabinoid concentration in serum versus blood.

Materials and Methods

1. Reagents and Supplies

The certified reference materials for Δ^9 -tetrahydrocannabinol (THC, 1 mg/mL in methanol), Δ^9 -tetrahydrocannabinol- d_3 (THC- d_3 , 1 mg/mL in methanol), cannabidiol (CBD, 1 mg/mL in methanol), cannabinol (CBN, 1 mg/mL in methanol), 11-hydroxy- Δ^9 -

tetrahydrocannabinol (11-OH-THC, 1 mg/mL in methanol), 11-hydroxy- Δ^9 -tetrahydrocannabinol- d_3 (11-OH-THC- d_3 , 100 μ g/mL in methanol), 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH, 1 mg/mL in methanol), and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol- d_9 (THC-COOH- d_9 , 1 mg/mL in methanol) were purchased from Cerilliant Corporation (Round Rock, TX, USA). HPLC-grade glacial acetic acid, ethyl acetate, deionized water (diH_2O), ammonium hydroxide, and N,O-Bis(trimethylsilyl)trifluoroacetamide (with 1% trimethylchlorosilane) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). HPLC-grade hexane and LC/MS-grade acetonitrile were purchased from Spectrum Chemical (New Brunswick, NJ, US).

Blank (drug-free) calf blood was obtained from O. Ottomanelli & Sons (New York, NY, US) for the preparation of the calibrator samples. Blank sheep serum was purchased from Hemostat Laboratories (Dixon, CA, US) for the preparation of the quality control (QC) samples. Hospital serum samples that previously screened negative for cannabinoids by the enzyme-linked immunosorbent assay (ELISA) were obtained from the NYC-OCME. Clean Screen Xcel II SPE columns were purchased from UCT, Inc. (Bristol, PA, US) and placed on a SPEware CEREX® System 48 processor for positive pressure SPE (Tecan, Männedorf, Switzerland). The eluent was evaporated under nitrogen using a SPEware CEREX® 48 Concentrator (Tecan, Männedorf, Switzerland).

2. Instrumental Parameters

The analysis of cannabinoids in serum by GC-MS/MS was performed using an Agilent 7890B GC System equipped with an autosampler and connected to an Agilent 7000C GC/MS Triple Quadrupole mass spectrometer (Agilent Technologies, Santa Clara,

CA, US). Chromatographic separation was accomplished using an Agilent DB17MS LTM column (15 m x 250 μm x 0.25 μm) and pre-column (1 m x 150 μm x 1.2 μm). The temperature of the LTM column started at 200°C and increased to 250°C over 6.5 min, remaining at 250°C for an additional minute of the run. Overall, the total run time was 9.5 min in which the GC system equilibrated back to initial conditions during the final two minutes of the run.

The triple quadrupole mass spectrometer analyzed the compounds using electron impact (EI) ionization mode. The data were collected using multiple reaction monitoring (MRM) mode and processed using Agilent MassHunter Quantitative Analysis for QQQ. The instrumental parameters for the five target analytes and three internal standards were previously optimized by the Department of Forensic Toxicology at the NYC-OCME (Table 1). 11-hydroxy- Δ^9 -tetrahydrocannabinol- d_3 (11-OH-THC- d_3) and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol- d_9 (THC-COOH- d_9) were employed as the deuterated internal standards for 11-hydroxy- Δ^9 -tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH), respectively. Δ^9 -tetrahydrocannabinol- d_3 (THC- d_3) served as the internal standard for Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN).

Table 1. MRM GC-MS/MS parameters for the derivatized target analytes and internal standards in serum (n = 8). The quantifier ion transitions are highlighted in bold.

Compound Name	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
CBD	4.2	390	301	15
			375	15
			319	15
THC	5	386	303	20
			330	10
			289	25
CBN	5.4	367	310	40
			323	35
			295	45
11-OH-THC	5.9	371	289	20
			305	15
			265	15
THC-COOH	6.8	371	289	15
		488	297	20
		488	371	20
THC- <i>d</i> ₃	5	389	306	20
			330	10
			292	25
11-OH-THC- <i>d</i> ₃	5.9	374	292	20
			308	15
			268	15
THC-COOH- <i>d</i> ₉	6.8	380	292	15
		497	306	20
		497	380	20

3. Preparation of the Calibrator and QC Samples

The working stock solution containing the five cannabinoids was prepared at 10 mg/L in methanol. This solution was serially diluted to final concentrations of 0.1 and 1 mg/L for a total of three working stock solutions for the calibrator samples. This procedure was repeated to create another set of working stock solutions for the QC samples. The internal standard solution containing the three deuterated internal standards was prepared at 0.5 mg/L in methanol. All these solutions were stored in a refrigerator at 5°C when not in use.

Table 2. Guidelines for the preparation of the calibrator and QC samples in 0.5 mL of biological sample (blood or serum).

Sample Type	Final Concentration (ng/mL)	Working Stock Solution (mg/L)	Volume Added (µL)
Calibrator	1	0.1	5
	5	0.1	25
	10	0.1	50
	25	1	12.5
	50	1	25
	80	1	40
	100	1	50
QC	3	0.1	15
	20	1	10
	70	1	35

The mixed mode anion exchange SPE procedure required 0.5 mL of blank calf blood or sheep serum to be aliquoted into labeled 16 x 125 mm glass culture tubes. The

calibrator (1, 5, 10, 25, 50, 80, and 100 ng/mL) and QC (3, 20, and 70 ng/mL) samples were prepared by spiking the appropriate working stock solution into 0.5 mL of blank calf blood or sheep serum, respectively. The appropriate volumes of working stock solution for the calibrator and QC samples are presented in Table 2.

4. Sample Extraction

The sample extraction procedure was based on the standard operating procedure (SOP) for the analysis of cannabinoids in blood by GC-MS/MS at the NYC-OCME. After fortifying the calibrator and QC samples with the appropriate volumes of working stock solution, 25 μ L of 0.5 mg/L internal standard solution was added to all the tubes for a final concentration of 25 ng/mL. The samples were immediately vortexed using the Scientific Industries Vortex Genie Z (Scientific Industries Inc., Bohemia, NY, USA). Protein precipitation involved adding 1.5 mL of ice-cold acetonitrile to each sample dropwise while vortexing, followed by centrifugation at 3,000 rpm for 10 min. The acetonitrile was decanted into labeled 10 mL conical test tubes and evaporated under nitrogen to approximately 200 μ L. After vortexing the samples, 2 mL of diH₂O was added to all the tubes and the samples were vortexed again. The samples were decanted onto UCT Clean Screen Xcel II solid phase extraction cartridges that were placed on a positive pressure manifold, and passed through the column at 1-2 psi. Subsequently, the columns were washed with 2 mL of diH₂O: acetonitrile: ammonium hydroxide (84:15:1, v/v) and dried for 10 min at 60 psi. The target analytes were eluted into labeled 16 x 125 mm glass culture tubes with 2 mL of hexane: ethyl acetate: glacial acetic acid (49:49:2, v/v) by gravity, and evaporated to dryness under nitrogen at room temperature. Then, the

samples were reconstituted with 50 μL of ethyl acetate and derivatized with 50 μL of BSTFA (with 1% TMCS). After capping and vortexing all the tubes, the samples were incubated for 30 min at 70° C. Finally, the derivatized extracts were transferred to labeled screw-capped vials with polymer feet inserts for GC-MS/MS analysis.

5. Method Cross-Validation

The cross-validation for the analysis of cannabinoids in serum by GC-MS/MS was performed using the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology (Scientific Working Group for Forensic Toxicology, 2013). The reference methodology was the determination of cannabinoids in whole blood by GC-MS/MS, which was previously developed and validated at the NYC-OCME Forensic Toxicology Laboratory. The following parameters were assessed: bias, imprecision, limit of detection (LOD), limit of quantitation (LOQ), interferences, extraction efficiency, dilution integrity, and stability. The other validation parameters of calibration model and carryover were previously evaluated during the validation of cannabinoids in blood by GC-MS/MS at the NYC-OCME.

a. Bias and Imprecision

Bias and imprecision were concurrently evaluated by monitoring QC samples at 3, 20, and 70 ng/mL over a course of five extractions with different calibration curves ($n = 15$). The maximum acceptable bias was not to exceed $\pm 20\%$ at each QC concentration. Similarly, the within-run and between-run precisions, which were calculated using the

one-way analysis of variation (ANOVA) approach, were not to exceed 20% at each QC concentration.

b. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were designated as the value of the lowest non-zero calibrator sample (1 ng/mL). Blank hospital serum samples from three different sources were analyzed using the criteria for detection, identification, bias, and imprecision. In order to meet the requirements for the LOD, the samples must produce a reproducible instrument response that is greater than 3x the signal-to-noise ratio (S/N) of the blank sample. For the LOQ, the samples must produce a reproducible instrument response that is greater than 10x the S/N of the blank sample, and result in bias not exceeding $\pm 20\%$ and imprecision less than 20%.

c. Interferences

Endogenous interferences were studied by extracting blank serum samples from ten different sources without the addition of internal standard solution. Interferences from the reference standards were studied by fortifying blank serum samples with either the target analytes at the upper limit of the calibration range (100 ng/mL) or the internal standard solution, both in triplicate. Interferences from high concentrations of CBD were investigated by extracting a 500 ng/mL sample of CBD and monitoring its possible conversion into THC.

d. Extraction Efficiency

Extraction efficiency was evaluated by comparing the area responses of five QC samples to ten blank serum extracts with post-extraction cannabinoid addition. Extraction efficiency was evaluated at the low (3 ng/mL) and high (70 ng/mL) QC concentrations.

e. Dilution Integrity

Dilution integrity was investigated by performing 1:2 and 1:5 dilution ratios of a 150 ng/mL sample, both in triplicate, and evaluating their effects on bias.

f. Stability

The stability of the derivatized extracts was evaluated by re-injecting the low and high QC samples on the autosampler after 24, 48, and 72 h at room temperature. The percent differences between the initial and re-injected QC samples were calculated in which the maximum acceptable percent difference was not to exceed $\pm 20\%$ at each QC concentration.

6. Identification and Quantification Criteria

The criteria that were used for the identification of target analytes consisted of the following: the retention time must be within $\pm 2\%$ of the average calibrator retention time, one quantifier and two qualifier product ions must be present, and the ion ratio qualifier/quantifier must be within $\pm 20\%$ of the average calibrator ion ratios. In order to quantify the target analytes, the calibration curve for each target analyte must have an r^2 value greater than 0.99. However, the NYC-OCME permits the exclusion of up to two

calibration points for a total of five calibration points to improve calibration curve linearity, calibrator or QC accuracy, or account for spiking or extraction issues in a specific calibrator sample.

7. Authentic Case Sample Analysis

The NYC-OCME Forensic Toxicology Laboratory provided serum (n = 20) and blood (n = 16) authentic case samples for the analysis of cannabinoids by GC-MS/MS. These samples were selected because they previously screened positive for cannabinoids by ELISA during initial testing. In addition, these samples were submitted between January and May of 2019, and stored in vacutainer glass collection tubes in a refrigerator at 5°C when not in use. The blood sample sources included femoral (n = 1) and hospital blood (n = 15). Among these serum and blood authentic case samples, there were eight paired blood and serum samples that were collected at the same time at the hospital. The manner of death for these paired blood and serum samples included three accidental deaths, two homicides, and three natural deaths. Furthermore, these eight paired blood and serum samples were used for the comparison of cannabinoid concentration in serum versus blood.

Results

1. GC-MS/MS Method Cross-Validation in Serum

The analysis of cannabinoids in serum by GC-MS/MS demonstrated excellent sensitivity and selectivity in which all the target analytes were detected and quantified within the calibration range of 1-100 ng/mL (Figure 1).

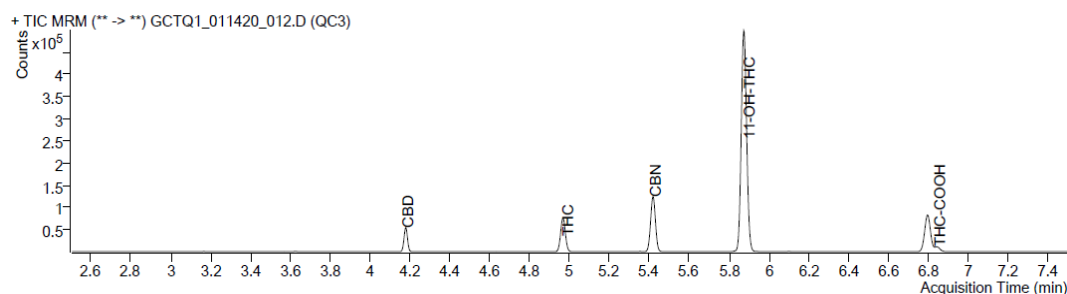


Figure 1. Total ion chromatogram (TIC) of a low QC (3 ng/mL) sample in sheep serum. The five target analytes eluted in the following order: CBD, THC, CBN, 11-OH-THC, and THC-COOH.

The cross-validation for cannabinoids in serum by GC-MS/MS was based on the previous validation for cannabinoids in blood by GC-MS/MS at the NYC-OCME. These methods were validated for the simultaneous quantification of five cannabinoids: THC, CBD, CBN, 11-OH-THC, and THC-COOH. The results from the cross-validation for cannabinoids in serum by GC-MS/MS are summarized in the following sections.

a. Bias and Imprecision

Bias and imprecision were concurrently evaluated by monitoring QC samples at the low (3 ng/mL), mid (20 ng/mL), and high (70 ng/mL) QC concentrations. All the target analytes showed acceptable bias ($\pm 20\%$) and imprecision ($< 20\%$), except for THC-COOH. Although THC-COOH failed the bias criteria with a bias of -27.6% at the mid QC concentration, it showed acceptable imprecision at each QC concentration. The bias and imprecision results for the target analytes in serum are presented in Tables 3 and 4, respectively.

Table 3. Summary of bias results at each QC concentration (3, 20, and 70 ng/mL) for the target analytes in serum (n = 15).

Compound Name	Bias (%)		
	<i>Low QC</i>	<i>Mid QC</i>	<i>High QC</i>
CBD	-17.1	-19.8	-18.1
THC	-7.9	-11.3	-11.3
CBN	-13.8	-14.9	-17.1
11-OH-THC	-8.5	-9.4	-8
THC-COOH	5.7	-27.6	7.4

Table 4. Summary of imprecision results at each QC concentration (3, 20, and 70 ng/mL) for the target analytes in serum (n = 15).

Compound Name	Low QC		Mid QC		High QC	
	<i>Intra-day %CV</i>	<i>Inter-day %CV</i>	<i>Intra-day %CV</i>	<i>Inter-day %CV</i>	<i>Intra-day %CV</i>	<i>Inter-day %CV</i>
CBD	9.6	5.5	9.6	6.1	10	6.4
THC	7.2	4	6.2	3.8	4.9	3.2
CBN	17.1	7.1	7.3	4	11.3	4.9
11-OH-THC	6.4	2.7	5.4	3.3	4.9	3.1
THC-COOH	12.4	5.3	14.4	7.9	6.9	3.1

b. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

At the LOD and LOQ (1 ng/mL), the bias results were within $\pm 20\%$ of the target range (0.8-1.2 ng/mL) and the imprecision results were less than 20% for all the target analytes (Table 5). In addition to satisfying the identification criteria, each target analyte produced a reproducible instrument response that was greater than 10x the S/N of the blank sample.

Table 5. Summary of bias and imprecision results at the LOD and LOQ (1 ng/mL) for the target analytes in serum (n = 9).

Compound Name	Bias (%)	Imprecision (%CV)	
		<i>Intra-day</i>	<i>Inter-day</i>
CBD	-7.2	12	16.6
THC	-4.8	9.7	16.5
CBN	5.2	5.4	9.7
11-OH-THC	-11.4	3.3	9.7
THC-COOH	2.3	14.9	19.5

c. Interferences

The presence of exogenous interferences from high concentrations of CBD was investigated by extracting a 500 ng/mL sample of CBD in triplicate. THC-COOH was detected in two replicates at 0.3 and 0.4 ng/mL. Similarly, THC-COOH was detected in all three replicates at concentrations ranging from 1.1-1.6 ng/mL during the validation of cannabinoids in blood by GC-MS/MS.

No matrix interferences were observed from the serum matrices used (n = 10). In addition, blank serum samples that were fortified with internal standard solution only did not produce positive results for the target analytes. However, 11-OH-THC-*d*₃ was detected in two replicates of the blank serum samples that were fortified with the target analytes at the upper limit of the calibration range (100 ng/mL). The response of 11-OH-THC-*d*₃ was approximately 2-3.5% of its response when compared to samples that were fortified with internal standard solution too. Similarly, 11-OH-THC-*d*₃ was detected in all three replicates during the validation of cannabinoids in blood by GC-MS/MS.

d. Extraction Efficiency

Extraction efficiency was evaluated by comparing the area responses of five QC samples to ten blank serum extracts with post-extraction cannabinoid addition. All the target analytes demonstrated optimal extraction efficiencies (> 50%), ranging from 51.3% for THC-COOH to 58.2% for CBN at the high QC (70 ng/mL) concentration (Table 6). However, the SPE procedure, poor derivatization, and protein precipitation step could have contributed to the reduced recovery of the target analytes.

Table 6. Summary of extraction efficiency results at the low (3 ng/mL) and high (70 ng/mL) QC concentrations for the target analytes in serum (n = 5).

Compound Name	Extraction Efficiency (%)	
	<i>Low QC</i>	<i>High QC</i>
CBD	53.9	56.5
THC	52.3	57.1
CBN	54.3	58.2
11-OH-THC	57.6	54.9
THC-COOH	57.5	51.3

e. Dilution Integrity

Dilution integrity was investigated by performing 1:2 and 1:5 dilution ratios of a 150 ng/mL sample, both in triplicate, and evaluating their effects on bias. When the dilution factors of 2 and 5 were applied on Agilent MassHunter Quantitative Analysis for QQQ, the average concentrations of CBD and CBN fell below the target range (120-180 ng/mL). In contrast, the average concentrations of THC, 11-OH-THC, and THC-COOH calculated within the target range (Table 7).

Table 7. Summary of dilution integrity results for the target analytes in serum (n = 3).

The concentrations should be within $\pm 20\%$ of the target concentration (120-180 ng/mL).

Compound Name	Average Concentration (ng/mL)	
	<i>1:2 Dilution Ratio</i>	<i>1:5 Dilution Ratio</i>
CBD	88.1	89.9
THC	123.4	123.1
CBN	104.9	108.2
11-OH-THC	126.4	124.3
THC-COOH	140.8	136

f. Stability

The stability of the derivatized extracts was evaluated by comparing the percent differences between the initial and re-injected low and high QC samples on the autosampler after 24, 48, and 72 h at room temperature. All the target analytes had percent differences less than 20%, except for THC. THC showed percent differences $\geq -21.4\%$ at the high QC concentration (70 ng/mL) after 24 h on the autosampler and $\geq -20.9\%$ at the low QC concentration (3 ng/mL) after 48 h on the autosampler. Therefore, samples that are on the autosampler for at least 24 h are not expected to provide consistent concentrations as compared to initial injections.

2. Comparison of Validation Parameters in Serum vs. Blood

All the validation parameters that were assessed during the cross-validation in serum were previously evaluated during the validation in blood by GC-MS/MS. Overall, the results were similar in both biological matrices. The following sections summarize

the main differences between the results from the cross-validation in serum and validation in blood by GC-MS/MS at the NYC-OCME.

a. Calibration Curve Linearity

The calibration models for the target analytes were previously established during the validation of cannabinoids in blood by GC-MS/MS at the NYC-OCME. The calibration models were evaluated within the range of 1-100 ng/mL by fortifying blank calf blood samples with the appropriate volumes of working stock solution over a course of five extractions. The calibration models that best fit the data by having the lowest standard deviation of residuals are summarized in Table 8.

Table 8. Linearity parameters for the target analytes in blood (n = 5).

Compound Name	Linear Range (ng/mL)	Weighting	Average R^2 Value	CV% of R^2 Values	Average Slope	CV% of Slopes
CBD	1-100	$1/x^2$	0.9928	0.2	6.8162	11
THC	1-100	$1/x$	0.997	0.3	0.9564	5.7
CBN	1-100	$1/x^2$	0.9962	0.2	31.428	5.8
11-OH-THC	1-100	$1/x$	0.9969	0.3	1.0137	5.9
THC-COOH	1-100	$1/x^2$	0.9947	0.3	1.1674	6.1

b. Bias and Imprecision

As depicted in Table 3, THC-COOH failed the bias criteria with a bias of -27.6% at the mid QC concentration (20 ng/mL) in serum. In contrast, all the target analytes

showed acceptable bias during the validation of cannabinoids in blood. In addition, the imprecision results for all the target analytes were similar in both biological matrices.

c. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

At the LOD and LOQ (1 ng/mL), the bias results were within the target range (0.8-1.2 ng/mL) and the imprecision results were less than 20% for all the target analytes in serum. In addition, each target analyte produced a reproducible instrument response that was greater than 10x the S/N of the blank sample. During the validation of cannabinoids in blood, THC-COOH failed the bias criteria with a bias of 34.3% but presented acceptable imprecision and S/N.

d. Extraction Efficiency

As depicted in Table 6, all the target analytes demonstrated extraction efficiencies (> 50%) at the low (3 ng/mL) and high (70 ng/mL) QC concentrations in serum. All the target analytes exhibited lower extraction efficiencies in blood, ranging from 22.8% for THC-COOH at the high QC concentration (70 ng/mL) to 45.4% for CBD at the low QC concentration (3 ng/mL). Although this validation parameter did not have specific pass or fail criteria, the results suggest that this extraction procedure recovers the target analytes more efficiently in serum than blood (Table 9).

Table 9. Comparison of extraction efficiency results at the low (3 ng/mL) and high (70 ng/mL) QC concentrations for the target analytes in blood and serum (n = 5).

Compound Name	Extraction Efficiency (%)			
	<i>Blood</i>		<i>Serum</i>	
	Low QC	High QC	Low QC	High QC
CBD	45.4	42.3	53.9	56.5
THC	36.2	35.3	52.3	57.1
CBN	33.9	35.1	54.3	58.2
11-OH-THC	35.2	32.9	57.6	54.9
THC-COOH	27.4	22.8	57.5	51.3

e. Dilution Integrity

The dilution integrity results suggest that sample dilution in blood provides more accurate and reliable quantitative results for all the target analytes. In contrast to the results presented in Table 7, the average concentrations for all the target analytes were within the target range (120-180 ng/mL) during the validation of cannabinoids in blood.

f. Stability

The stability results indicate that the derivatized extracts were more stable in blood than serum when left on the autosampler after given time intervals at room temperature. During the validation of cannabinoids in blood, all the target analytes had percent differences within $\pm 20\%$, except for CBN with a percent difference of 20.9% at the low QC concentration (3 ng/mL) after 72 h on the autosampler.

3. Authentic Case Sample Analysis

Overall, 20 serum samples and 16 blood samples were analyzed for cannabinoids by GC-MS/MS. The most commonly detected target analyte was THC-COOH, which was detected in all the serum and blood samples. THC and 11-OH-THC were detected in one serum sample and six blood samples, CBD was detected in one blood sample, and CBN was not detected in either biological matrix. Figures 2 and 3 illustrate the overall trend of the target analytes being more frequently detected in blood than serum.

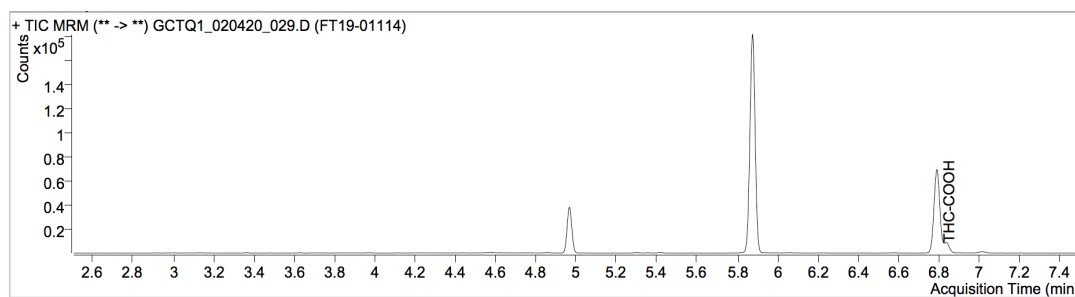


Figure 2. Total ion chromatogram (TIC) of serum sample from case number 7. THC-COOH (3.2 ng/mL) was detected.

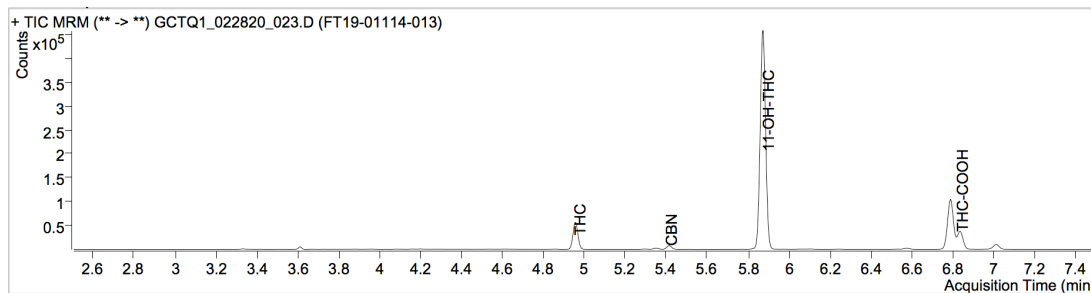


Figure 3. Total ion chromatogram (TIC) of hospital blood sample from case number 7. THC (1.8 ng/mL), 11-OH-THC (1.4 ng/mL), and THC-COOH (8.8 ng/mL) were detected.

Table 10 provides the range and median concentrations for the target analytes that were detected in the serum (n = 20) and blood (n = 16) authentic case samples. Target analytes that were detected at concentrations less than the LOD and LOQ (1 ng/mL) were not included in these results.

Table 10. Summary of target analyte concentrations in blood (n = 16) and serum (n = 20) authentic case samples.

Compound Name	Statistics	Blood (ng/mL)		Serum (ng/mL)
		<i>Femoral</i>	<i>Hospital</i>	
CBD	Range	1.2	-----	-----
	Median			
	# of Cases	1		
THC	Range	4.6	1.8 – 7.7	2.5
	Median		2	
	# of Cases	1	5	
CBN	-----	-----	-----	-----
11-OH-THC	Range	1.8	1.3 – 3.2	1.5
	Median		2.1	
	# of Cases	1	5	
THC-COOH	Range	>100	2.5 – >100	2 – >100
	Median		21.1	9
	# of Cases	1	15	20

Furthermore, the ratio of THC-COOH concentration in blood to serum was calculated for each paired blood and serum authentic case (n = 8). The average blood-to-serum ratio was 2.1, which indicates that THC-COOH was generally detected at higher concentrations in blood than serum (Table 11). However, this ratio was less than 1 for

three paired blood and serum authentic cases, which demonstrates that THC-COOH was detected at higher concentrations in serum than blood too. As illustrated in Figure 4, no strong correlation was observed between the different matrix concentrations for THC-COOH.

Table 11. Ratio of THC-COOH concentration in blood to serum from paired blood and serum authentic cases (n = 8).

Statistics	Blood-to-Serum Ratio
Mean	2.1
Standard Deviation	1.8
Range	0.4 – 5.6

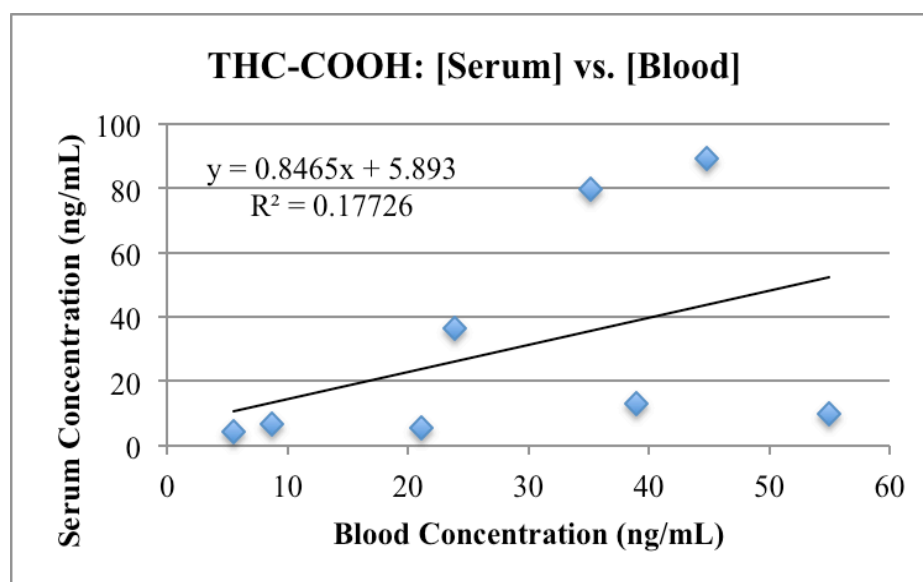


Figure 4. Scatter plot of THC-COOH concentration in serum versus blood from paired blood and serum authentic cases (n = 8).

Discussion

Compared to the methods that are currently published in the literature, the present method requires a small volume of serum sample (0.5 mL) and encompasses a comprehensive working range of 1-100 ng/mL for these five target analytes (Citti et al., 2018; Gottardo et al., 2019). All the target analytes presented acceptable bias and imprecision, except for THC-COOH at the mid QC concentration (20 ng/mL). This is not considered a major concern for the quantification of THC-COOH because its bias results were within $\pm 20\%$ at the other QC concentrations, its precision results were less than 20%, and it did not affect the other validation parameters. Although some methods utilize lower LOD and LOQ values, this is not critical because the cut-offs for initial and confirmatory testing of cannabinoids in blood require analytical results as low as 1 ng/mL, which is the value of the LOD and LOQ for the present method.

The interferences that resulted from fortifying blank serum samples with either a high concentration of CBD or the target analytes at the upper limit of the calibration range (100 ng/mL) were also present during the validation of cannabinoids in blood by GC-MS/MS, and are not expected to interfere with the assay. The dilution integrity results suggest that sample dilution in blood provides more accurate and reliable quantitative results for all the target analytes. In addition, the stability results suggest that THC is unstable in serum when left on the autosampler at room temperature. In contrast, studies have reported that THC remains stable in serum when left on the autosampler after 24 h (Gasse et al., 2016; Purschke et al., 2016). Nonetheless, the stability results for THC were very close to the maximum acceptable percent difference ($\pm 20\%$). Perhaps, refrigeration or rederivatization of the processed samples could improve the stability of

THC (Andrenyak et al., 2017). Furthermore, all the target analytes demonstrated greater extraction efficiencies in serum than blood.

Although THC-COOH was detected in all the serum and blood authentic case samples, the other target analytes were more commonly detected in blood than serum. The ratio of THC-COOH concentration in blood to serum was calculated for the eight paired blood and serum authentic cases to identify a correlation between the different biological matrices. Similarly, Giroud et al. (2001) calculated the ratios of THC, 11-OH-THC, and THC-COOH concentrations in plasma or serum to whole blood for eight paired plasma and whole blood cases and six paired postmortem blood and serum cases. Their results were similar in that THC-COOH was detected in all the samples, followed by THC and then 11-OH-THC. However, they concluded that these cannabinoids were detected at higher concentrations in plasma and serum than whole blood.

Moreover, studies have concluded that cannabinoid concentrations are greater in serum than whole blood due to high plasma protein binding and poor cannabinoid distribution in erythrocytes (Giroud et al., 2001; Schwilke et al., 2009; Urfer et al., 2014). The current project produced mixed results in which the ratio of THC-COOH concentration in blood to serum ranged from 0.4-5.6. Further research with a larger sample size is necessary to elucidate cannabinoid distribution in blood and serum. Other important parameters that should be considered include storage temperature and the type of collection tubes used for the hospital serum and blood samples. These factors may influence the stability of cannabinoids in biological samples, and therefore explain the differences in the obtained results.

Conclusion

The cross-validation for cannabinoids in serum by GC-MS/MS was performed based on the previous validation for cannabinoids in blood by GC-MS/MS at the NYC-OCME. These methods involved SPE followed by GCTQ for the simultaneous quantification of CBD, THC, CBN, 11-OH-THC, and THC-COOH. In addition, they were applied to serum (n = 20) and blood (n = 16) authentic case samples obtained from the NYC-OCME to investigate cannabinoid distribution in serum and blood. Lastly, the present method is important for forensic cases that require a high degree of sensitivity and selectivity for the determination of cannabinoids in serum.

References

- Andrenyak, D. M., Moody, D.E., Slawson, M. H., O’Leary, D. S., & Haney, M. (2017). Determination of Δ -9-tetrahydrocannabinol (THC), 11-hydroxy-THC, 11-nor-9-carboxy-THC and cannabidiol in human plasma using gas chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology*, *41*(4), 277-288.
doi:10.1093/jat/bkw136
- Castro, A. L., Tarelho, S., Melo, P., & Franco, J. M. (2018). A fast and reliable method for quantitation of THC and its 2 main metabolites in whole blood by GC-MS/MS (TQD). *Forensic Science International*, *289*, 344-351.
doi:10.1016/j.forsciint.2018.06.003
- Citti, C., Braghiroli, D., Vandelli, M. A., & Cannazza, G. (2018). Pharmaceutical and biomedical analysis of cannabinoids: A critical review. *Journal of Pharmaceutical and Biomedical Analysis*, *147*, 565-579.
doi:10.1016/j.jpba.2017.06.003
- Compton, R. (2017). *Marijuana-impaired driving – A report to Congress* (DOT HS 812 440). Retrieved from
<https://www.nhtsa.gov/sites/nhtsa.dot.gov/files/documents/812440-marijuana-impaired-driving-report-to-congress.pdf>
- Gasse, A., Pfeiffer, H., Köhler, H., & Schürenkamp, J. (2016). Development and validation of a solid-phase extraction method using anion exchange sorbent for the analysis of cannabinoids in plasma and serum by gas chromatography-mass spectrometry. *International Journal of Legal Medicine*, *130*(4), 967-974.
doi:10.1007/s00414-016-1368-6

- Giroud, C., Ménétreay, A., Augsburger, M., Buclin, T., Sanchez-Mazas, P., & Mangin, P. (2001). Δ^9 -THC, 11-OH- Δ^9 -THC and Δ^9 -THCCOOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people. *Forensic Science International*, *123*(2-3), 159-164.
doi:10.1016/s0379-0738(01)00538-2
- Gottardo, R., Sorio, D., Ballotari, M., & Tagliaro, F. (2019). First application of atmospheric-pressure chemical ionization gas chromatography tandem mass spectrometry to the determination of cannabinoids in serum. *Journal of Chromatography A*, *1591*, 147-154. doi:10.1016/j.chroma.2019.01.041
- Hartman, R. L., Richman, J. E., Hayes, C. E., & Huestis, M. A. (2016). Drug recognition expert (DRE) examination characteristics of cannabis impairment. *Accident Analysis & Prevention*, *92*, 219-229. doi:10.1016/j.aap.2016.04.012
- Kicman, A.T. & King, L.A. (2014). The current situation with cannabinoids. *Drug Testing and Analysis*, *6*(1-2), 1-6. doi:10.1002/dta.1597
- Lacroix, C. & Sausseureau, E. (2012). Fast liquid chromatography/tandem mass spectrometry determination of cannabinoids in micro volume blood samples after dabsyl derivatization. *Journal of Chromatography B*, *905*, 85-95.
doi:10.1016/j.jchromb.2012.08.006
- Levine, B. (2003). Principles of forensic toxicology. Washington, DC: AACC Press.
- Levinsohn, E. A. & Hill, K. P. (2020). Clinical uses of cannabis and cannabinoids in the United States. *Journal of the Neurological Sciences*, *411*, 1-6.
doi:10.1016/j.jns.2020.116717
- Marcoux, R., Holmes, L., & Vogenberg, F. R. (2019). Regulatory and legislative

- disparities with cannabis present challenges to P&T committees and health care providers. *Pharmacy and Therapeutics*, 44(5), 290–293.
- Nahar, L., Guo, M., & Sarker, S. D. (2019). Gas chromatographic analysis of naturally occurring cannabinoids: A review of literature published during the past decade. *Phytochemical Analysis*, 31(2), 135-146. doi:10.1002/pca.2886
- Negrusz, A. & Cooper, G. (2013). Clarke's analytical forensic toxicology. London, UK: Pharmaceutical Press.
- Peterman, D. R. (2019). *Marijuana use and highway safety* (R45719). Retrieved from <https://crsreports.congress.gov>
- Purschke, K., Heinl, S., Lerch, O., Erdmann, F., & Veit, F. (2016). Development and validation of an automated liquid-liquid extraction GC/MS method for the determination of THC, 11-OH-THC, and free THC-carboxylic acid (THC-COOH) from blood serum. *Analytical and Bioanalytical Chemistry*, 408(16), 4379-4388. doi:10.1007/s00216-016-9537-5
- Raikos, N., Schmid, H., Nussbaumer, S., Ambach, L., Lanz, S., Längin, A., ... Weinmann, W. (2014). Determination of Δ^9 -tetrahydrocannabinolic acid A (Δ^9 -THCA-A) in whole blood and plasma by LC-MS/MS and application in authentic samples from drivers suspected of driving under the influence of cannabis. *Forensic Science International*, 243, 130-136. doi:10.1016/j.forsciint.2014.07.026
- Schaefer, N., Kettner, M., Laschke, M. W., Schlote, J., Peters, B., Bregel, D., ... Schmidt, P. H. (2015). Simultaneous LC-MS/MS determination of JWH-210, RCS-4, Δ^9 -tetrahydrocannabinol, and their main metabolites in pig and human serum, whole blood, and urine for comparing pharmacokinetic data. *Analytical and*

Bioanalytical Chemistry, 407(13), 3775-3786. doi:10.1007/s00216-015-8605-6

Schwilke, E. W., Karschner, E. L., Lowe, R. H., Gordon, A. M., Cadet, J. L., Herning, R. I., & Huestis, M. A. (2009). Intra- and intersubject whole blood/plasma cannabinoid ratios determined by 2-dimensional, electron impact GC-MS with cryofocusing. *Clinical Chemistry*, 55(6), 1188-1195.
doi:10.1373/clinchem.2008.114405

Schwoppe, D. M., Scheidweiler, K. B., & Huestis, M. A. (2011). Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 401(4), 1273-1283. doi:10.1007/s00216-011-5197-7

Scientific Working Group for Forensic Toxicology. (2013). Scientific working group for forensic toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, 37(7), 452-474.
doi:10.1093/jat/bkt054

Substance Abuse and Mental Health Services Administration. (2019). *Key substance use and mental health indicators in the United States: Results from the 2018 National Survey on Drug Use and Health* (HHS Publication No. PEP19-5068, NSDUH Series H-54). Retrieved from <https://www.samhsa.gov/data/>

Urfer, S., Morton, J., Beall, V., Feldmann, J., & Gunesch, J. (2014). Analysis of Δ^9 -tetrahydrocannabinol driving under the influence of drugs cases in Colorado from January 2011 to February 2014. *Journal of Analytical Toxicology*, 38(8), 575-581.
doi:10.1093/jat/bku089

Appendix

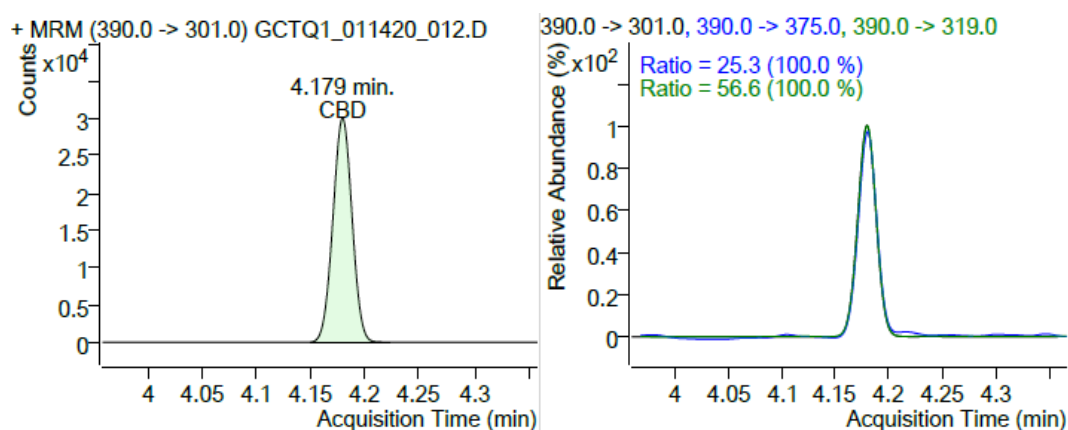
Appendix A: Acronyms and Abbreviations

11-OH-THC	11-hydroxy- Δ 9-tetrahydrocannabinol
ANOVA	Analysis of Variance
CBD	Cannabidiol
CBN	Cannabinol
CV	Coefficient of Variation
DUID	Driving Under the Influence of Drugs
EI	Electron Impact
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
GCTQ	Gas Chromatography Coupled to Triple Quadrupole Tandem Mass Spectrometry
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantitation
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
NYC-OCME	New York City Office of Chief Medical Examiner
QC	Quality Control

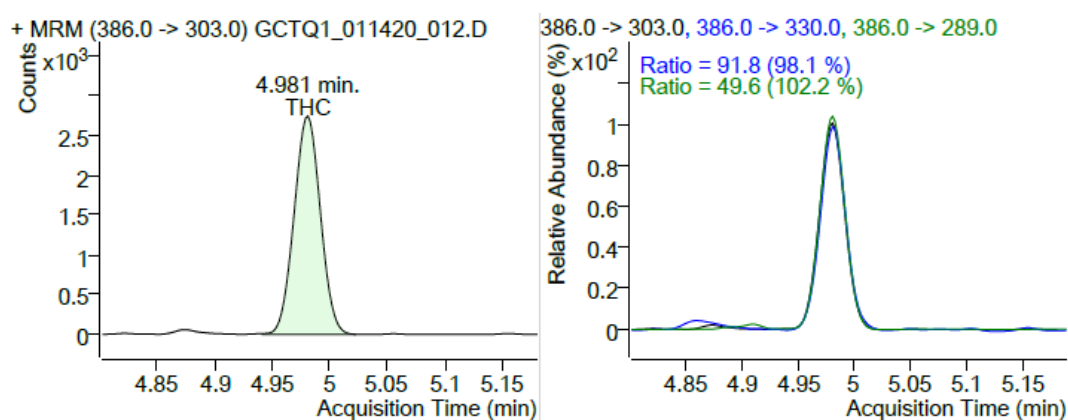
S/N	Signal-to-Noise
SOP	Standard Operating Procedure
SPE	Solid Phase Extraction
SWGTOX	Scientific Working Group for Forensic Toxicology
THC	Δ 9-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy- Δ 9-tetrahydrocannabinol
TIC	Total Ion Chromatogram

Appendix B: Extracted Ion Chromatograms of Target Analytes at the Low QC (3 ng/mL)

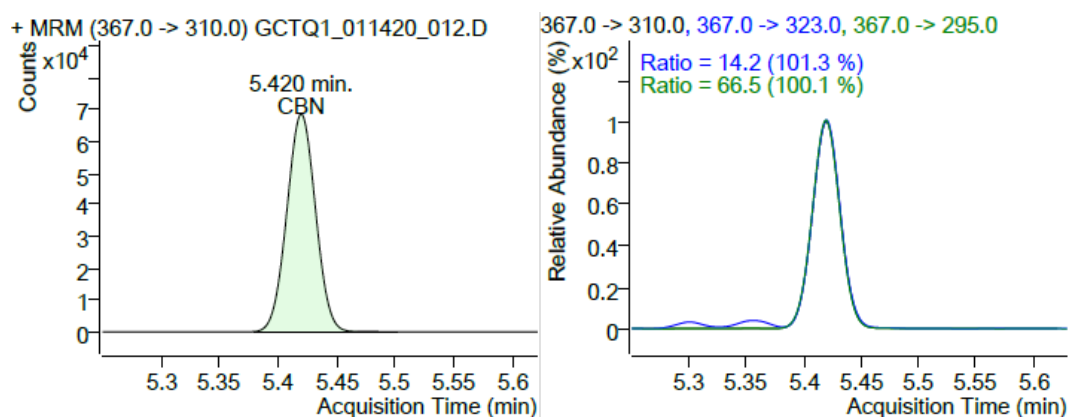
CBD



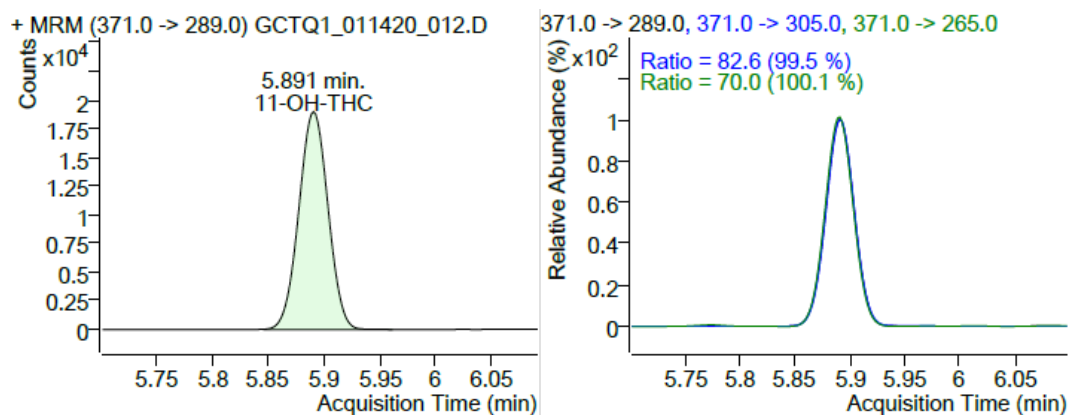
THC



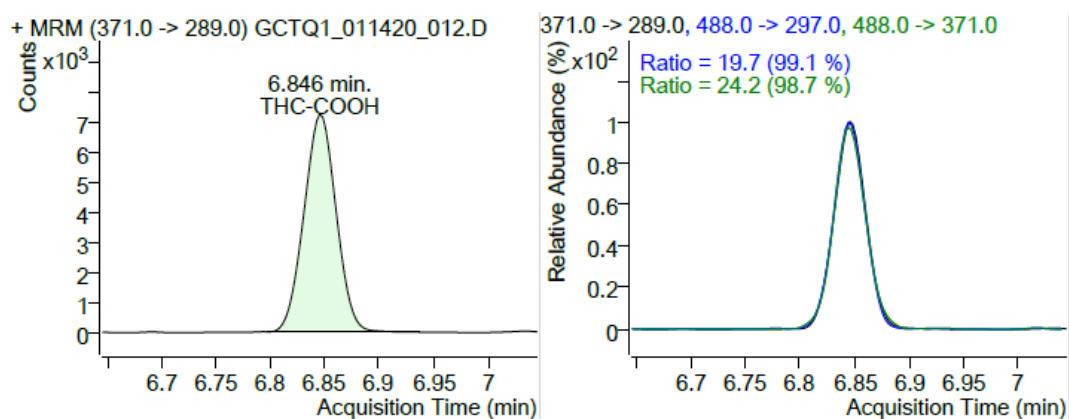
CBN



11-OH-THC



THC-COOH



Appendix C: Results for Blood (n = 16) and Serum (n = 20) Authentic Case Samples

Case Number	Compound Name	Concentration (ng/mL)		Source of Blood Tested	Additional Comments
		<i>Blood</i>	<i>Serum</i>		
1	THC	7.7	Not Detected	Hospital Blood	
	11-OH-THC	3.2	Not Detected		
	THC-COOH	38.9	12.9		
2	THC	Not Detected	2.5	Hospital Blood	
	11-OH-THC	Not Detected	1.5		
	THC-COOH	44.8	89.1		
3	THC-COOH	> 100	28.9	Hospital Blood	Suicide
4	THC-COOH	N/A	1.9	N/A	
5	THC-COOH	2.5	4.4	Hospital Blood	
6	THC-COOH	N/A	6.3	N/A	
7	THC	1.8	Not Detected	Hospital Blood	
	11-OH-THC	1.4	Not Detected		
	THC-COOH	8.8	3.2		
8	THC-COOH	4.9	3.4	Hospital Blood	
9	THC-COOH	3.4	9.6	Hospital Blood	
10	THC-COOH	35.1	79.7	Hospital Blood	
11	CBD	1.2	Not Detected	Femoral	
	THC	4.6	Not Detected		
	11-OH-THC	1.8	Not Detected		
	THC-COOH	> 100	> 100		
12	THC-COOH	N/A	8.5	N/A	
13	THC-COOH	N/A	28.4	N/A	
14	THC	2.3	Not Detected	Hospital Blood	
	11-OH-THC	2.1	Not Detected		

	THC-COOH	47.6	32.4		
15	THC-COOH	21.1	5.3	Hospital Blood	
16	THC-COOH	5.5	4.2	Hospital Blood	
17	THC-COOH	16.4	4.9	Hospital Blood	
18	THC	1.9	Not Detected	Hospital Blood	
	11-OH-THC	1.3	Not Detected		
	THC-COOH	23.9	36.6		
19	THC-COOH	8.7	6.7	Hospital Blood	
20	THC	2	Not Detected	Hospital Blood	
	11-OH-THC	2.2	Not Detected		
	THC-COOH	54.9	9.8		