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Postmortem Fluid and Tissue Concentrations of THC, 11-OH-THC, and THC-COOH

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16. Abstract

Marijuana is the most commonly abused illicit drug worldwide. Marijuana is used for its euphoric and relaxing properties. However, marijuana use has been shown to result in impaired memory, cognitive skills, and psychomotor function. The Federal Aviation Administration's Civil Aerospace Medical Institute conducts toxicological analysis on aviation fatalities. Due to severe trauma associated with aviation accidents, blood is not always available; therefore, the laboratory must rely on specimens other than blood for toxicological analysis in approximately 30-40% of cases. However, the postmortem distribution of cannabinoids has not been well characterized. The purpose of this research is to evaluate the distribution of THC, and its metabolites 11-OH-THC, and THC-COOH in postmortem fluid and tissue specimens from 11 fatal aviation accident cases (2014-2015) previously found positive for cannabinoids. Specimens evaluated, when available, included: blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, heart, and bile. We developed and validated (following SWGTOX guidelines) a sensitive and robust method using solid phase extraction and liquid chromatography-tandem mass spectrometry to identify and quantify THC, 11-OH-THC and THC-COOH in postmortem fluids and tissues. The method readily identified and quantified these cannabinoids in postmortem fluids and tissues below 1 ng/mL. Qualitative cannabinoid results within each case were comparable between blood and non-blood specimens. However, there was no consistent distribution of the cannabinoids between blood and any other fluids or tissues. Therefore, while quantitative interpretation of non-blood specimens is not prudent, a majority of the non-blood specimens tested could be suitable alternative/supplemental choices for qualitative cannabinoid purposes.

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POSTMORTEM FLUID AND TISSUE CONCENTRATIONS OF THC, 11-OH-THC, AND THC-COOH

INTRODUCTION

Approximately 147 million individuals worldwide currently use marijuana, making it the most widely used drug of abuse.(1) A survey conducted in 2010 showed that 6.9% of individuals 12 and older in the United States used marijuana in the preceding month.(2) With an increasing number of states now legalizing marijuana, both recreationally and medicinally, marijuana use is on the rise.(3)

The primary psychoactive compound in marijuana is Δ^9-tetrahydrocannabinol (THC). Once introduced to the body, THC is rapidly metabolized to an equipotent psychoactive short-term metabolite, 11-hydroxy-tetrahydrocannabinol (11-OH-THC), which is then metabolized to the inactive metabolite 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH).(4) THC is typically used for its euphoric effects.(4) Non-desirable side effects include dizziness, dry mouth, increased heart rate, and impaired memory, concentration, and reaction time.(4) Numerous studies have shown that THC has detrimental effects on cognitive and psychomotor function and skills.(5-11) Moreover, operation of a motor vehicle under the influence of marijuana has been found to have similar effects as in individual with a blood alcohol content of 0.08%.(12) Another study compared ethanol ingestion with marijuana smoking; they found a similar degree of both perceived impairment and actual impairment.(5) Aircraft simulator studies have shown that following the use of marijuana, pilots deviated in altitude and had trouble following flight patterns compared to when they were "sober."(13-16)

Following toxicological evaluation of a fatality, toxicologists are expected to form educated opinions about the postmortem findings. However, there is minimal postmortem fluid and tissue data in the literature relating to THC, 11-OH-THC, and THC-COOH. Furthermore, the analysis of postmortem tissues for THC, 11-OH-THC, and THC-COOH is not common in the forensic community. Our laboratory currently utilizes solid-phase extraction (SPE) and gas chromatography with negative chemical ionization mass spectrometry (GC/MS-NCI) for the analysis of THC and THC-COOH in postmortem fluids and tissues.(17) While our current method is suitable for the analysis of postmortem fluids and tissues for THC and THC-COOH, there are a few limitations with this method: our extraction requires 3 mL of fluid specimen, GC/MS analysis requires multiple time-consuming derivatizations, GC/MS-NCI relies on retention time and the M-H ion only for identification, and it does not detect 11-OH-THC.

In order to have a method that uses a smaller sample size, eliminates time consuming derivatizations, and identifies/quantitates 11-OH-THC (a short-lasting metabolite of THC), we determined that we needed to utilize liquid chromatography-tandem mass spectrometry (LC/MS/MS). There are numerous LC/MS/MS methods in the literature for the identification and quantitation of THC and its metabolites; however, few analyzed THC, 11-OH-THC, and

THC-COOH in postmortem fluids and tissues. Furthermore, there have been a limited number of studies published on the distribution of these cannabinoids in postmortem fluids and tissues.(17-19) This paper details the validation and application of SPE coupled with LC/MS/MS for the identification and quantitation of THC, 11-OH-THC, and THC-COOH in postmortem fluid and tissue specimen from 11 aviation accident fatalities.

MATERIALS AND METHODS

Chemicals and Reagents

THC, 11-OH-THC, and THC-COOH methanolic standards were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX) at 1.00 mg/mL. THC-d_3, 11-OH-THC-d_3, THC-COOH-d_3, THC-glucuronide, and THC-COOH-glucuronide methanolic standards were purchased from Cerilliant at 100 µg/mL. Selectrazyme (abalone derived β-glucuronidase) was obtained from UCT (UCT, Inc., Bristol, PA). Sodium acetate and potassium oxalate were purchased from Sigma-Aldrich (Sigma-Aldrich; St. Louis, MO). Formic acid, sodium fluoride, hydrochloric acid, hexanes, ethyl acetate, and LC/MS grade acetonitrile, isopropanol, and methanol were purchased from Fisher (Fisher Scientific, Pittsburgh, PA). Double deionized (DI) water was obtained from a Millipore Direct Q-3 UV (Millipore, Continental Water Systems, El Paso, TX). Bovine blood was obtained from Country Home Meat Co. (Country Home Meat Co., Edmond, OK). Immediately upon collection, sodium fluoride and potassium oxalate were added to the blood and mixed to produce a final sodium fluoride/potassium oxalate concentration of 1.0% and 0.2% (w/v), respectively.

Mobile phase A (MPA) was made with DI water with formic acid (999:1 v/v). Mobile phase B (MPB) was made with LC/MS grade acetonitrile with formic acid (999:1 v/v). A seal wash solution at a 25:25:25:25 (v/v) ratio was prepared with DI water, and LC/MS grade acetonitrile, methanol, and isopropanol.

Sample selection and storage

The toxicology laboratory's database (ToxFLO, DiscoverSoft Development, LLC; Oklahoma City, OK) was searched for cases that were previously found positive for cannabinoids within the last 2 years (2014 – 2015) that had blood and a majority of the desired specimens available for analysis (urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, heart, and bile). Blood samples were stored in gray top tubes (1.0% and 0.2% (w/v) sodium fluoride/potassium oxalate, respectively). All specimens were stored at -20°C until analysis.

Ultra-performance liquid-chromatography-tandem mass spectrometric conditions

Analysis was performed on a Waters® Xevo TQ-S Acquity Ultra Performance Liquid Chromatography (UPLC) (Waters Corporation; Milford, MA). The column manager temperature was set at 30°C. Acquity UPLC BEH C18 column (2.1 x 100 mm, 1.7 µm) was used for chromatographic separation. Prior to analysis, the column was equilibrated for 30 min. The UPLC was operated with a gradient of 35:65 MPA:MPB (MPA - water with 0.1% formic acid;

MPB - acetonitrile with 0.1% formic acid) to 10:90 MPA:MPB at 2.0 min, held until 2.5 min, and then returned to 35:65 MPA:MPB at 3.8 min and held until 4.0 min for a total run time of 4.0 min. The UPLC flow rate was 0.60 mL/min and a sample injection volume was 2 µL. The autosampler was set at 10°C. UPLC pressures observed for these conditions were around 9,000 psi. Following use, the column was washed and stored in acetonitrile.

All compounds were optimized on the mass spectrometer by direct infusion. Four different modes of compound ionization were evaluated (positive and negative atmospheric pressure chemical ionization - ±APCI, and positive and negative electrospray ionization - ±ESI) and the +ESI mode provided maximum ionization for the compounds. The source temperature was set at 150°C, desolvation temperature at 550°C, and desolvation gas flow at 1,100 L/hr. Precursor (parent) and product (daughter) ions, quantitation ions, cone voltage, collision energy, and ion mode are listed in Table 1.

Table 1. MS parameters for THC, 11-OH-THC, and THC-COOH.

Compound	Retention Time	Cone Voltage (V)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
THC	2.43	38.0	315.26	123.12 193.19*	32.0 22.0
THC-d_3	2.41	19.0	318.30	123.11 196.24*	33.0 21.0
11-OH-THC	1.47	20.0	331.25	193.19* 313.32	25.0 13.0
11-OH-THC-d_3	1.46	20.0	334.30	196.24* 316.30	23.0 13.0
THC-COOH	1.51	20.0	345.23	299.29 327.31*	19.0 15.0
THC-COOH-d_3	1.49	20.0	348.30	302.29 330.31*	19.0 15.0

*Transition ion used for quantification.

There are three criteria set by our laboratory that must be met before an analyte can be reported as positive by LC/MS/MS. An analyte's product ions must have a minimum signal-to-noise ratio of 10 (quant ion) and 3 (confirmation ion), a retention time +/- 5% of the average calibrator retention time, and product ion ratio +/- 20% of the average calibrator product ion ratio.

Calibration and control preparation

All calibrators and controls were prepared using bovine whole blood. Calibrators and controls were prepared using separate 1.00 mg/mL methanolic drug standards. Calibration curves consisted of eight calibrators and were prepared by serial dilution to produce concentrations ranging from 0.78 to 100 ng/mL. A 100 ng/mL internal standard solution was prepared in DI water using 100 µg/mL THC-d_3, 11-OH-THC-d_3, and THC-COOH-d_3 methanolic standards. Controls were prepared at concentrations of 2, 20, and 80 ng/mL, covering low, medium, and

high portions of the calibration curve. Controls were used to determine the accuracy and precision of the method, both within-day and between-day, and analyte stability. Controls were analyzed with all case specimens to ensure the calibration curve accuracy.

THC, 11-OH-THC, and THC-COOH are present in urine, to some degree, as glucuronide conjugates. Therefore, we hydrolyzed each postmortem urine specimen using the enzyme *β*-glucuronidase (Selectrazyme, 100,000 units/mL, UCT; Bristol, PA). This solution was stored in a refrigerator at 4°C. THC and THC-COOH glucuronide controls, prepared as 25 ng/mL free drug, were made from a 100 µg/mL methanolic standards of THC-glucuronide and THC-COOH-glucuronide. The 11-OH-THC-glucuronide standard was unavailable.

Quantification was achieved via an internal standard calibration procedure. Response factors for each compound were determined for every sample analyzed. The response factor was calculated by dividing the area of the analyte quant ion peak by the area of the internal standard quant ion peak. Calibration curves were derived by plotting the analyte/internal standard response factor versus the analyte concentration for each respective calibrator and determining the mathematical model that best fit the calibration data. These calibration curves were then used to determine the concentrations of each cannabinoid compound in the prepared controls and biological specimens.

Sample preparation and extraction method

All calibrators, controls, and specimens were prepared using the following procedure. Tissue samples were diluted 2:1 w:w (1% NaF solution:tissue) and then homogenized using an OMNI homogenizer (Omni International; Kennesaw, GA). One-half mL of liquid specimen and 1.5 g of tissue homogenate (0.5 g of wet tissue) were added to individual 16 x 100 mm screw-top tubes. Each specimen was spiked with 100 µL of the 100 ng/mL internal standard solution (10 ng total). Samples were vortexed briefly and allowed to stand at room temperature for 10 min. Fifty µL of stock Selectrazyme (10,000 units), followed by 2.00 mL of 1.0 M pH 5.00 sodium acetate buffer was added to a 16x150 screw top tube containing 0.5 mL urine sample. The urine samples were then vortexed briefly and incubated at 65°C for 3 h to facilitate hydrolysis of all glucuronide conjugates. In our initial investigations, complete hydrolysis of both THC and THC-COOH-glucuronide conjugates was achieved after incubation with Selectrazyme for 2.5 h at 65°C (unpublished data). However, an incubation time of 3 h was chosen to ensure that specimens with elevated cannabinoid concentrations were also completely hydrolyzed. Following hydrolysis, samples were allowed to cool to room temperature. Three mL of ice-cold acetonitrile was added to all specimens (except urine specimens) and the specimens were immediately vortexed for 30 sec. Samples were capped, rotated for 5 min, and centrifuged for 10 min at 2300 rpm in a Thermo Jouan C4i Centrifuge (Thermo Electron Corp.; Chateau-Gontier, France). The supernatant was transferred to a clean 16x100 mm round bottom tube and 5 mL of 0.1 M Sodium Acetate Buffer pH 3 was added.

Buffered samples were transferred to the SPE Clean Screen THC columns that were preconditioned with 2 mL of methanol, 2 mL of DI water, and 1 mL of 0.1 M Sodium Acetate Buffer pH 3. The Clean Screen THC columns, 10 mL barrel, 200 mg sorbent bed, were obtained from UCT (UCT; Bristol, PA). After the sample passed through the column, the columns were washed with 2 mL of DI water, 2 mL of 95:5 100mm HCl:ACN, and 200 µL of hexanes. Samples were then eluted into a 16x100 mm conical tube. The first elution was 2 mL of hexanes (for THC). The second elution was 2 mL 50:50 hexanes:ethyl acetate (for 11-OH-THC and THC-COOH). Elutions were taken to dryness in a TurboVap LV nitrogen evaporator (Caliper Life Sciences; Hopkinton, MA) set at 40°C, reconstituted in 100 µL of LC/MS grade methanol, and transferred to 2 mL LC vials with inserts. The vials were capped and transferred to the auto sampler.

RESULTS AND DISCUSSION

Analytical Method

This LC/MS/MS method proved to be sensitive, robust, and reproducible for the detection and quantitation of THC, 11-OH-THC, and THC-COOH. The UPLC provided sharp, narrow analyte peaks which were far superior to traditional HPLC. A typical chromatogram can be seen in Figure 1. This method was validated following SWGTOX guidelines.(20) These guidelines recommend the evaluation of calibration curve best fit model, carryover, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, dilution integrity, stability, ion suppression/enhancement, and drug interference for the validation of a new analytical method.

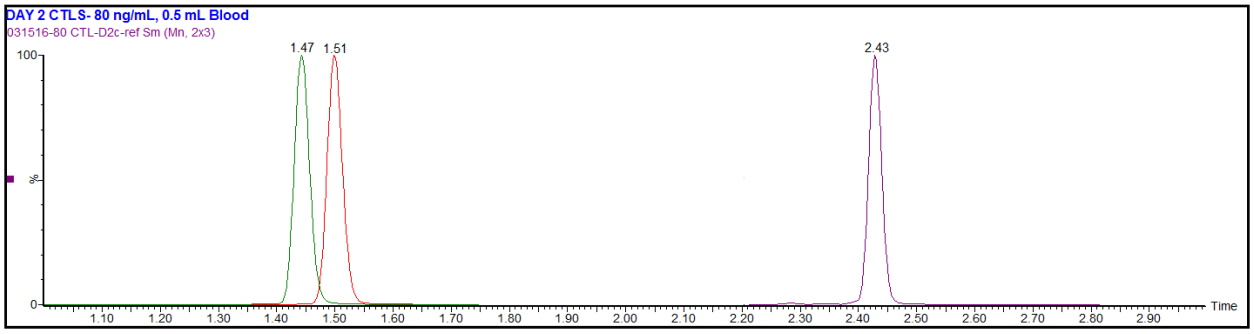

Figure 1. An 80 ng/mL control with 11-OH-THC, THC-COOH, and THC (left to right).

The calibration curve best fit model was determined by preparing and analyzing a calibration curve on 5 separate, but consecutive days. The THC, 11-OH-THC, and THC-COOH calibration curves were constructed of concentrations ranging from 0.78– 100 ng/mL. All curves best fit a linear regression model with a 1/x weighting. The linear dynamic range (LDR) for THC, 11-OH-THC, and THC-COOH was found to be 0.78 – 100 ng/mL. Correlation coefficients were all greater than 0.99.

LOD is the lowest analyte concentration meeting all identification criteria described in the materials and methods section, i.e., minimum signal-to-noise ratio of 10 for quantitation ion and

3 for all other ions, retention time +/- 5% of the average calibrator retention time, and product ion ratio +/- 20% of the average calibrator product ion ratio. The LOQ has the same identification requirements, plus it must have a measured concentration +/- 20% of target or expected concentration. The lowest calibrator tested (0.78 ng/mL) met the qualitative and quantitative criteria for THC, 11-OH-THC, and THC-COOH. Since concentrations below this were not tested, the LOD and LOQ were administratively determined to be 0.78 ng/mL. This method could readily determine concentrations far below the lowest calibrator (0.78 ng/mL), but we determined this was a low enough concentration for our laboratory's needs and mission. All case specimens falling below the LOD/LOQ were considered negative.

Carryover was evaluated for all analytes; no carryover was observed. Carryover was evaluated by running a methanol blank after the highest calibrator during the linearity study described above. The blanks showed no trace of THC, 11-OH-THC, or THC-COOH. Carryover was also monitored throughout the study by including blanks between specimens to verify that no carryover occurred.

Method accuracy was determined by calculating the difference between the target concentration and the determined concentration and is expressed as bias or relative error (%E). Precision is expressed as the coefficient of variation (CV) of multiple analyses at a given control level. Controls at 2, 20, and 80 ng/mL of each analyte were prepared on Day 1 of the accuracy, precision, and stability study. Controls were prepared in sufficient quantities (100 mL bovine blood) for use in all studies and were aliquoted into 16x100 mm screw-top tubes. Freshly prepared controls were extracted on Day 1 of the study. The remainder of the controls were stored either in the refrigerator (4°C) or in the freezer (-20°C) until use.

The Day 1 accuracy and precision data demonstrate that this method is very accurate and precise. The largest Day 1 %E for all THC, 11-OH-THC, and THC-COOH control levels were -9, -8, and -14, respectively. The largest CV for all THC, 11-OH-THC, and THC-COOH control levels were 3, 8, and 2, respectively. On Days 2, 3, 4, and 5, controls were removed from the refrigerator and analyzed with a freshly prepared calibration curve. The within-day %E for all controls were less than +/- 20% and the CV was less than 10%, a SWGTOX recommendation. The grand mean, or between-day, accuracy and precision also demonstrated small %E and CV. The accuracy and precision data is summarized in Tables 2, 3, and 4.

All three cannabinoids examined here exist to some degree in urine as a glucuronide conjugate. In order to determine total THC, 11-OH-THC and THC-COOH, the glucuronide bond must be cleaved. This is best achieved enzymatically, using a beta-glucuronidase hydrolysis step. To ensure this step provided a quantitatively reliable hydrolysis, a THC and THC-COOH glucuronide control was analyzed with all urine specimens. No 11-OH-THC-glucuronide was commercially available, so no control for this analyte was used. The target concentration for each glucuronide control was 25 ng/mL free-drug. The control values were within 20% of the target concentration.

Actual specimens may have cannabinoid concentrations that exceed the highest calibrator in the calibration curve. Since measured values must be within the LDR, concentrations outside the LDR cannot be reported and, thus, a specimen dilution may be necessary. Therefore, we evaluated the dilution integrity of the cannabinoids. Five 80 ng/mL controls were diluted 1:10 and five 1 µg/mL controls were diluted 1:100. All dilutions were within 20% of the expected dilution concentration of 8 ng/mL and 100 ng/mL.

Stability was evaluated in three ways: on-instrument stability (post-extraction stability), refrigerated stability, and freeze/thaw stability. On-instrument stability or post-extraction stability was determined by reanalyzing (re-injecting) all three levels of the Day 1 controls on Days 2, 3, 4, and 5. Specimens were left on the instrument in the autosampler that was set at 10°C. All three compounds were found to be stable for five days post-extraction. Refrigeration stability was evaluated by extracting controls stored at refrigerator temperatures on Days 2, 3, 4, and 5. This data is the same as the accuracy and precision data described above. It was found that all three analytes were stable for five days in the refrigerator at 4°C. We also evaluated freeze-thaw stability. In order to determine if THC, 11-OH-THC and THC-COOH are stable after multiple freeze/thaw cycles, controls were placed in a freezer at -20°C on Day 1. On Day 2, all frozen controls were removed from the freezer and allowed to thaw for one hour at room temperature. Five specimens at each control concentration were analyzed and the remainders of the controls were placed back into the freezer. Three freeze/thaw cycles were completed. It was found that all three analytes were stable after three freeze/thaw cycles.

Ion suppression/enhancement was determined by analyzing multiple analyte-spiked solvent samples and spiked post-extraction fluid and tissue specimens and comparing their response. If the post-extraction specimen had a lower signal than the solvent control sample, it is ion suppression. If it has a higher signal, then it is ion enhancement. Ion suppression/enhancement was evaluated in blood, urine, serum, liver, lung, brain, and muscle. Ion suppression was not a factor for THC, 11-OH-THC, or THC-COOH in blood or urine specimens (<20%). THC and 11-OH-THC also suffered no suppression in serum (<20%). THC-COOH did experience approximately 40% ion suppression in serum. Additionally, significant ion suppression was observed in tissue specimens for all 3 analytes. However, similar ion suppression also affected the deuterated internal standards, thus the quantification of these analytes in these specimens was not affected.

It is common to encounter multiple drugs in a typical case. Therefore, drug interference needed to be evaluated to determine if common drugs can alter the detection and/or quantitation of THC, 11-OH-THC, or THC-COOH. Drugs commonly encountered in our laboratory were prepared at final concentration of 5 µg/mL with 80 ng/mL cannabinoids. These drugs included acetaminophen, atenolol, atorvastatin, citalopram, dextromethorphan, diphenhydramine, hydrocodone, methamphetamine, naproxen, and sertraline. Five of these controls were analyzed and none suffered qualitative or quantitative interference.

Table 2. Accuracy and Precision for THC.

DAY 1				DAY 2			DAY 3			DAY 4			DAY 5					
Target (ng/mL)	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Grand Mean (ng/mL)	CV%	E%
2	1.83 ± 0.02	1	-9	1.69 ± 0.02	1	-16	1.73 ± 0.04	2	-13	1.73 ± 0.05	3	-14	1.69 ± 0.01	1	-15	1.73 ± 0.06	3	-14
20	21.4 ± 0.2	1	7	20.4 ± 0.2	1	1	19.5 ± 0.3	1	-3	22.7 ± 0.2	1	13	23.8 ± 0.5	2	19	22 ± 2	9	10
80	81 ± 2	3	1	80 ± 1	2	1	76.5 ± 0.5	1	-5	88.6 ± 0.9	1	11	95 ± 1	1	19	84 ± 7	8	5

n=5 for all measurements
Accuracy measured as relative error (%E) from target concentration.
Precision measured as CV in replicate measurements.

Table 3. Accuracy and Precision for 11-OH-THC.

DAY 1				DAY 2			DAY 3			DAY 4			DAY 5					
Target (ng/mL)	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Grand Mean (ng/mL)	CV%	E%
2	1.8 ± 0.2	8	-8	1.72 ± 0.02	1	-14	1.68 ± 0.09	6	-16	1.9 ± 0.1	6	-3	1.79 ± 0.06	3	-11	1.8 ± 0.2	11	-10
20	20.5 ± 0.4	2	3	19.4 ± 0.6	3	-3	17.8 ± 0.5	3	-11	20.3 ± 0.9	4	1	19.2 ± 0.2	3	-4	20.5 ± 0.4	2	3
80	80 ± 2	3	0	82 ± 2	2	3	73 ± 2	3	-8	81.5 ± 1	1	2	84 ± 1	1	5	80 ± 2	3	0

n=5 for all measurements
Accuracy measured as relative error (%E) from target concentration.
Precision measured as CV in replicate measurements.

Table 4. Accuracy and Precision for THC-COOH.

DAY 1				DAY 2			DAY 3			DAY 4			DAY 5					
Target (ng/mL)	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Mean (ng/mL)	CV%	E%	Grand Mean (ng/mL)	CV%	E%
2	1.72 ± 0.04	2	-14	1.79 ± 0.08	4	-11	1.83 ± 0.07	4	-8	1.72 ± 0.04	2	-14	1.86 ± 0.08	4	-7	1.78 ± 0.08	4	-11
20	19.8 ± 0.3	2	-1	21.5 ± 0.6	3	7	20.8 ± 0.3	1	4	21.0 ± 0.3	2	5	22 ± 1	5	11	21 ± 1	5	5
80	79.9 ± 0.7	1	0	85 ± 1	1	7	85 ± 1	2	6	88 ± 2	2	10	87 ± 2	2	9	85 ± 3	4	6

n=5 for all measurements
Accuracy measured as relative error (%E) from target concentration.
Precision measured as CV in replicate measurements.

Postmortem specimen analysis

Postmortem toxicology evaluation is an essential part of aviation accident investigation. The most desired specimen for postmortem toxicology is blood. However, due to the nature of aviation accidents, blood is not always available for analysis. In fact, our laboratory relies exclusively on tissues in approximately 30 – 40% of our cases. Therefore, having the ability to reliably analyze postmortem tissues is important. Approximately 4% of aviation fatalities in 2015-2016 were positive for marijuana use. Understanding the postmortem distribution of cannabinoids could potentially aid in the interpretation of postmortem cannabinoid results. Eleven aviation fatalities (22 – 69 years of age, median 47 years old; all male) were analyzed with this newly validated method. These cases were previously confirmed and quantitated using GC/MS-NCI for THC and THC-COOH.

The distribution of THC, 11-OH-THC, and THC-COOH throughout various postmortem specimens is presented in Tables 5, 6, and 7. Blood specimens from all 11 cases were positive for THC-COOH, with concentrations ranging from 2.37 – 70.2 ng/mL. Eight of the 11 blood specimens were positive for THC, with concentrations ranging from 0.78 – 8.39 ng/mL. Only four of these THC-positive bloods had measurable concentrations of 11-OH-THC (1.03 – 4.59 ng/mL). The analysis of 11-OH-THC is important as it is a short-lasting metabolite of THC, which has been suggested to be an indicator of recent THC exposure.

Multiple non-blood specimens were analyzed to evaluate their possible usefulness as supplemental or alternative specimens for the identification and quantification of THC, 11-OH-THC, and THC-COOH including urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, heart, and bile. Results can be seen in tables 5, 6, and 7.

Ten of the 11 cases tested had non-blood fluids available for analysis (either urine, vitreous, and/or bile). Of the 8 cases with available urine, 7 were positive for THC (1.25 – 32.2 ng/mL). 11-OH-THC and THC-COOH were detected in all of the cases where urine specimens were available for analysis. The concentration of 11-OH-THC ranged from 11.7 – 620 ng/mL, and the THC-COOH ranged from 24.2 – 970 ng/mL. The case with the highest THC concentration also had the highest 11-OH-THC and THC-COOH concentrations. THC was detected in bile in 5 of the 9 cases containing bile (0.78 – 50.4 ng/mL). 11-OH-THC (0.98 – 227 ng/mL) and THC-COOH (201 – 3,307 ng/mL) were detected in all bile specimens. Bile yielded the highest THC-COOH concentrations of all the specimens tested. Due to excretory nature of bile, these elevated concentrations were not surprising. There were only two cases that had vitreous humor available for analysis. Neither case was positive THC or 11-OH-THC, while 1 case showed positive for THC-COOH.

Table 5. THC fluid and tissue concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
1	Neg	1.25	—	Neg	Neg	Neg	0.78	5.21	Neg	Neg	0.78
2	4.03	—	—	Neg	14.1	22.6	1.31	7.37	3.71	7.40	14.2
3	0.78	1.26	—	Neg	1.82	1.91	Neg	2.43	Neg	Neg	Neg
4	1.78	1.43	Neg	52.2	143	450	16.9	81.4	43.6	472	50.4
5	3.45	Neg	—	Neg	42.6	32.3	5.14	11.9	—	1.70	6.35
6	Neg	1.35	Neg	Neg	Neg	Neg	Neg	5.31	Neg	6.58	Neg
7	4.67	32.2	—	22.3	151	16.4	20.0	377	30.6	158	—
8	1.50	—	—	Neg	48.1	9.08	0.96	1.19	1.34	5.35	Neg
9	5.00	—	—	Neg	62.6	5.21	5.58	3.24	16.4	54.7	—
10	Neg	3.04	—	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
11	8.39	3.62	—	Neg	Unsuit	0.99	6.39	3.10	8.80	Neg	5.60

***** All concentrations shown in units of ng/mL or ng/g
— Specimen type not available for analysis

Table 6. 11-OH-THC fluid and tissue concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
1	Neg	13.5	—	Neg	Neg	Neg	Neg	Neg	Neg	Neg	5.13
2	Neg	—	—	Neg	Neg	Neg	Neg	Neg	3.31	Neg	21.2
3	Neg	13.4	—	Neg	Neg	Neg	Neg	Neg	Neg	Neg	16.8
4	2.02	60.6	Neg	66.1	12.7	17.9	19.8	10.9	37.4	29.9	227
5	Neg	43.3	—	4.04	1.15	1.34	Neg	Neg	—	Neg	20.6
6	Neg	11.7	Neg	1.27	Neg	Neg	Neg	Neg	Neg	Neg	0.98
7	4.59	620	—	38.6	6.95	10.5	13.8	12.0	30.8	8.6	—
8	Neg	—	—	Neg	Neg	Neg	Neg	Neg	0.99	Neg	1.93
9	1.03	—	—	Neg	2.18	1.77	Neg	Neg	8.97	Neg	—
10	Neg	53.4	—	Neg	Neg	Neg	Neg	Neg	Neg	Neg	32.4
11	1.74	25.9	—	8.17	Neg	Neg	Neg	Neg	3.01	Neg	61.7

***** All concentrations shown in units of ng/mL or ng/g
— Specimen type not available for analysis

Table 7. THC-COOH fluid and tissue concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
1	2.37	24.2	—	28.5	1.81	12.1	2.06	0.83	Neg	Neg	291
2	52.3	—	—	59.9	7.93	47.4	7.54	Neg	4.66	Neg	1000
3	3.13	109	—	29.1	3.11	10.1	Neg	0.80	0.98	Neg	565
4	68.0	102	2.7	3894	205	1774	284	66.8	73.4	346	3307
5	32.0	219	—	352	30.7	226	25.8	7.23	—	Neg	2238
6	2.62	87.6	Neg	57.3	1.31	33.6	2.83	1.17	2.52	3.32	201
7	70.2	970	—	2238	31.3	1482	62.9	27.0	28.8	59.6	—
8	4.60	—	—	77.0	2.66	45.6	4.63	1.76	2.54	3.16	310
9	9.32	—	—	46.3	5.94	107	10.5	0.87	7.51	Neg	—
10	3.17	200	—	114	14.3	28.0	16.9	Neg	3.39	Neg	485
11	65.7	189	—	529	50.0	222	15.7	10.2	12.3	Neg	301

***** All concentrations shown in units of ng/mL or ng/g
— Specimen type not available for analysis

All 11 cases had liver available for analysis. THC-COOH was detected in all liver samples (28.5 – 3,894 ng/g). THC was only detected in two cases (22.3 and 52.2 ng/g). Five liver specimens were positive for 11-OH-THC (1.27 – 66.1 ng/g). Two cases with the highest concentrations of 11-OH-THC were the same two cases where THC was detected. The liver contains the enzyme cytochrome P450, which is responsible for the metabolism of THC to 11-OH-THC and further to THC-COOH.(21) This could explain why THC was identified in only 2 liver specimens and 11-OH-THC was detected in 5 of the 11 livers.(22) While liver tissue does not show a large percentage of THC positives relative to other tissues, it may be a good alternative or supplemental specimen for cannabinoid testing because of the percentage of specimens positive for 11-OH-THC.

Lung was found to contain some of the highest concentrations of THC. Seven of the 11 lungs tested were found positive for THC (1.82 – 151 ng/g), while one was found to be unsuitable for analysis. 11-OH-THC was detected in four lung specimens (1.15 – 12.7 ng/g), primarily in cases with the higher THC values. THC-COOH was detected in all lung specimens (1.31 – 205 ng/g). The lung specimens with the highest THC concentration also had the highest concentration of THC-COOH. The high THC concentrations in this tissue may be due to the fact that the most common route of administration of THC is through the smoking of marijuana. Therefore, the lung is exposed to very high amounts of THC through the inhalation of marijuana smoke. Lung tissue appears to be a good alternative matrix for cannabinoid testing since it has a high number of positives and the cannabinoids are at high concentrations.

THC-COOH was positive in all kidney specimens (10.1 – 1,774 ng/g) at high concentration relative to most other tissue specimens. High concentrations of THC-COOH in the kidney were also found by Kemp et al. and Brunet et al.(17,23) THC was found in 8 of 11 kidneys studied (0.99 – 450 ng/g). Four cases were positive for 11-OH-THC (1.34 – 17.9 ng/g). Kidney tissue showed high concentrations of THC and THC-COOH, which may be due to the function of the kidney. Kidney tissue also appears to be a good alternative matrix for cannabinoid testing with its high cannabinoid concentrations.

Spleen and muscle also proved to be viable matrices for cannabinoid testing. THC was detected in 8 of 11 spleen specimens and 10 out of 11 muscle specimens. THC-COOH was detected in 10 of 11 spleen cases and 9 of 11 available muscles. The two cases with the highest THC and THC-COOH spleen and muscle concentrations were the only cases positive for 11-OH-THC.

The brain is a highly perfused organ and THC is quickly able to cross the blood-brain barrier. The detection of cannabinoids in the brain, even if they were absent in the blood agreed with a study conducted by Mura et al.(24) THC was detected in 6 of 10 brain tissues (1.34 – 43.6 ng/g). Each brain specimen that had THC also contained 11-OH-THC (0.99 – 37.4 ng/g). Nine of 10 brain specimens were positive for THC-COOH (0.98 – 73.4 ng/g). While brain did not have the highest concentration or largest percentage of THC positives, it did have the highest percentage

of 11-OH-THC positives of any specimen. Therefore, brain could also be a suitable supplemental specimen for cannabinoid analysis if 11-OH-THC is of interest.

Seven of the 11 heart specimens were positive for THC (1.70 – 472 ng/g) and four for THC-COOH (3.16 – 346 ng/g). Only two heart tissues showed 11-OH-THC (8.60 and 29.9 ng/g). These two heart specimens were the ones that had the highest concentration of THC and THC-COOH. With the low number of cannabinoid positive in this specimen type, heart does not appear to be as good of choice for an alternative matrix for cannabinoid analysis as are some of the other tissues.

We also examined the postmortem distribution of THC, 11-OH-THC, and THC-COOH for each specimen type to determine if there was a consistent distribution between non-blood specimens and blood (non-blood specimen concentrations divided by the blood concentration). No consistent distribution was identified for any of the cannabinoids tested. There are numerous reasons why there could be a lack of consistent distribution within a given tissue type. When the body dies, postmortem changes begin instantly.(25-29) Cells begin degrading and postmortem redistribution can occur. The degree of postmortem redistribution may vary based on sampling site and the postmortem interval.(30) A study that examined postmortem redistribution of cannabinoids between central and peripheral blood observed a trend between postmortem interval and increased postmortem redistribution.(31) The postmortem intervals for the cases in this study are unknown.

The blood collection site can also impact the apparent distribution when attempting to evaluate the distribution of a drug relative to its blood concentration. In a majority of the cases our laboratory receives for analysis, the blood collection site is not noted. When the collection site is reported, the blood typically is noted as having been collected from the chest cavity. Chest cavity blood is likely not representative of “true” blood, as it may be contaminated with fluids from the surrounding tissues and/or the stomach. Further, cannabinoid concentrations in cavity fluid could be affected by redistribution from surrounding tissues of higher cannabinoid concentrations, i.e., lung. In addition, cannabinoid concentrations in tissue specimens could vary based on the portion of tissue that is provided to our laboratory for analysis.

Individual characteristics such as gender, body composition, hepatic, and renal function could influence how the analytes distribute through the body. Since THC is lipophilic, individuals with high BMI, could have more THC stored in adipose tissue relative to other organs. There are many factors concerning the cannabis itself that could affect the distribution. Since these are postmortem cases, much information regarding the individual’s cannabis intake is unknown, including time of last use, how often they used cannabis, the potency, and if they smoked marijuana or consumed the THC orally by pill or in an edible.

Cannabinoid concentrations in the body will vary based on how often the individual used cannabis. Studies show that chronic users could have a concentration of THC and THC-COOH in their blood after 7 days of not using cannabis.(32) This could mean that the person smoked

marijuana several days before death and it was just still able to be detected in the fluid and tissue specimens.

Route of administration and the concentration of THC introduced to the body could affect the concentration in the body and where analytes are detected at higher levels. For example, if an individual was to eat “pot brownies,” the concentration of THC may not be as high in various tissues as it would if the individual would have smoked marijuana. This is because of first-pass metabolism, where much of the THC is metabolized in the liver to 11-OH-THC prior to circulation throughout the body.(21) However, in this scenario more THC may actually be consumed than during smoking. This is because ingesting marijuana takes longer for effects to be felt, so an individual could consume more marijuana trying to obtain the desired euphoric effect.

CONCLUSION

A rapid, sensitive, and robust method for the confirmation and quantitation of THC, 11-OH-THC, and THC-COOH in was developed using SPE and LC/MS/MS. This new method utilizes significantly less sample and is far less time consuming than our current GC/MS procedure. The LDR for all three cannabinoids was 0.78 – 100 ng/mL, covering the expected concentrations of these analytes. This validated method was successfully applied to 11 aviation fatalities to determine the concentrations of THC, 11-OH-THC, and THC-COOH in postmortem blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, heart, and bile. No consistent distribution within any specimen type was identified. Urine, bile and a majority of the tissue specimens tested appear to be good alternative specimens for qualitative cannabinoid testing. However, quantitatively the concentrations are relatively ambiguous, as little postmortem cannabinoids distribution data exists and the fact that postmortem specimens in general are not ideal due to postmortem changes. Postmortem concentrations should always be interpreted with a great deal of caution. This data should add to the limited information about cannabinoid distribution in postmortem fluids and tissues.

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