Document Title:	**Differences in Cannabis Impairment and its Measurement Due to Route of Administration**
Author(s):	**Megan Grabenauer**
Document Number:	**255884**
Date Received:	**December 2020**
Award Number:	**2016-DN-BX-0193**

Differences in Cannabis Impairment and its Measurement Due to Route of Administration

Final Summary Overview

Submitted to:
U.S. Department of Justice
Office of Justice Programs
National Institute of Justice
810 Seventh St., NW
Washington, DC 20531

Prepared by:
Megan Grabenauer (PI)
RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

March 31, 2020
Administrative Point of Contact:
Jackie Wilson
jwilson@rti.org
Phone: 919-541-5865
NIJ Award No. 2016-DN-BX-0193
RTI Project No. 0215514

Contents

Purpose

Current laws regarding cannabis impairment are either difficult to prosecute or are controversial. Currently, most law enforcement use a combination of biological and behavioral assessments administered by drug recognition experts (DREs) and blood THC levels, with cutoffs ranging from 1 to 5 ng/mL, to judge cases of suspected Driving Under the Influence of Drugs (DUID) involving cannabis. However, the behavioral assessments have not been explicitly developed to be sensitive for detecting acute intoxication from cannabis, and there are significant limitations to the use of blood THC levels as a proxy for acute intoxication. Also, though smoking remains the most common route of cannabis administration, cannabis is increasingly available in a wide array of "edibles" intended for oral ingestion and there has been a substantial increase in the use of vaporizers to inhale cannabis products. The purpose of this project was to better define the pharmacokinetics and associated pharmacodynamics of cannabis administered via vaporization and oral consumption in order to evaluate methods of determining whether or not an individual under the influence of cannabis is impaired.

Project Design

The project consisted of a comprehensive evaluation of acute dose effects for cannabis administered via vaporization and oral administration. This was achieved using a combination of behavioral and performance evaluations and forensic toxicology testing (blood, urine, and oral fluid) following controlled administration of known doses of cannabis.

Clinical Dosing Sessions

Clinical dosing sessions were completed at Johns Hopkins University (Baltimore, MD) under approved Institutional Review Board protocols for research with human subjects. Twenty individuals who had not used cannabis for at least 30 days participated in six, double-blind, experimental sessions each that were separated by at least one week. Across all six sessions each

participant consumed cannabis brownies containing 0 (placebo), 10, or 25 mg THC or inhaled vaporized cannabis containing 0 (placebo), 5, or 20 mg THC. Samples of blood, oral fluid, and urine were collected during each session. Subjective, cognitive, and psychomotor effects were assessed before cannabis administration (baseline) and for 8 hours thereafter at 0, 1, 2, 3, 4, 5, 6, and 8 hours after all doses. Oral fluid was collected at baseline, 0, 1, 2, 3, 4, 5, 6, and 8 hours after all doses. Urine was collected at baseline, 1, 2, 3, 4, 5, 6, and 8 hours after all doses. Blood was collected at baseline, 0, 1, 2, 3, 4, 6, and 8 hours after vaping doses and baseline, 1, 2, 3, 4, 5, 6, and 8 hours after oral doses. Cognitive and psychomotor tests administered included the paced serial addition test (PSAT), digit symbol substitution test (DSST), divided attention test (DAT), and tasks 1-4 from the DRUID iOS app. In addition, several field sobriety tests were administered including one leg stand, walk and turn, modified Romberg balance, and eye tracking for nystagmus and pupillary response.

Biofluid Analysis

Blood, oral fluid, and urine samples were sent to commercial laboratories for targeted LC-MS/MS analysis. Blood and oral fluid were analyzed by Immunalysis (Pomona, CA). Urine was analyzed by Clinical Reference Laboratory (Lenexa, KS). Analytes targeted for each matrix were as follows: Blood – THC, THC-COOH, 11-OH-THC, CBD, and CBN; Oral fluid – THC, THC-COOH, CBD, and CBN; Urine –THC, THC-COOH, delta8-THC, delta8-THC-COOH, THCV, THCV-COOH, 8-OH-THC, 11-OH-THC, 8,11-diOH-THC, CBD, and CBN.

Blood samples also underwent an exploratory screen at RTI for possible new biomarkers of impairment using non-targeted high-resolution mass spectrometry. The extraction method for the non-targeted assay was intentionally generic in order to not bias results. Blood (250 µL) was combined with 1,000 µL of acetonitrile containing aripiprazole-d8, dexthorphan-d3, doxepin-d3, and phenobarbital as internal standards. Samples were thoroughly mixed then centrifuged at

3,220 RCF for 5 min. The supernatant was dried under nitrogen at 40 ˚C and reconstituted in starting UHPLC mobile phase composition (90:10 H_2O:15% methanol in acetonitrile) prior to reversed phase UHPLC using a Waters HSS-T3 column (1.8 µm, 2.1x100 mm). Samples were analyzed using a Waters Synapt QTOF using the MS^E acquisition mode.

Eye Tracking

Eye movements were recorded using a DAX evidence recorder (Ocular Data Systems, Pasadena, CA) that was stationary on a desk in front of the participants. For all tests, the videos were first cropped to isolate each eye, and each eye was analyzed separately. For each frame, intensity values were adjusted such that 50% of the data was saturated at the highest intensity values, a bounding box was applied and the image thresholded, and pupil location was estimated based on number and location of dark pixels. After completing center tracking for both eyes, a time series of the x-center coordinates was formed, and the median value subtracted such that the signal was approximately centered around zero. Values above and below a certain threshold were removed (i.e., these were times when a feature such as the eyelashes were confused with the pupil). Then, blocks of 10 or more pixels where the x-center coordinate did not change were removed (i.e., this occurred during blink events and other times when the algorithm was not able to correctly identify the pupil).

Results

Subjective

Subjective drug effects were generally dose-orderly within each route of administration with peak effects being lower and delayed after oral ingestion compared to vaporized cannabis inhalation. Peak subjective effects generally occurred between 3-5 hours after oral dosing and 0-1 hour after vaped dosing. The THC doses administered mainly produced positive effects and were not unpleasant to participants.

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

Cognitive and Psychomotor

Working memory (PSAT), psychomotor functioning (DSST), and divided attention (DAT) were all negatively impacted after use of the high oral (25 mg THC) and vaporized (20 mg THC) doses. Oral dosing of 10 and 25 mg, and 20 mg vaporized THC doses impaired cognitive and psychomotor performance, but 5 mg vaporized cannabis produced discriminative drug effects with minimal impairment. After vaping, working memory (PSAT) and balance were affected immediately, whereas psychomotor functioning (DSST) and divided attention (DAT) performance were not significantly impacted until 1 hour after dosing. Peak effects were generally seen between 0 and 2 hours post dosing and performance returned to baseline levels by the 4 hour timepoint. After oral administration, cognitive and psychomotor performance were not impacted until 1 hour after dosing. Peak effects were generally seen around 5 hours post dosing (except for balance - which had a peak effect at 3 hours), and it remained elevated at the 6 hour timepoint, and returned to near baseline performance levels by 8 hours post dosing. One leg stand, walk and turn, and modified Romberg balance field sobriety tests, which are part of a battery of tests administered to detect alcohol impairment, were not sensitive to cannabis intoxication. Each field sobriety test is scored based on whether pre-defined clues are observed during the test. For example, stepping off the line or taking an incorrect number of steps for the walk and turn, or hopping or putting foot down during the one leg stand. There was no apparent difference in the rate of clue detection between oral administration and vaporized cannabis for any of the field sobriety tests.

Biofluid

Pharmacokinetic measures indicate target compound profiles are dose-orderly and route dependent. Target compound profiles in blood and oral fluid were similar. For both matrices, THC, CBD, and CBN were higher after vaping than after oral consumption. Conversely, THC-

COOH and 11-OH-THC were higher after oral consumption than vaping. Much higher levels of CBD and CBN were seen in oral fluid than in blood. Very high levels of THC were detected in oral fluid immediately after dosing for both routes of administration. Some of this is likely due to contamination of the mouth by the dose itself. Of all the analytes tested in oral fluid, THC had the highest concentrations after both routes of administration. Of all the analytes tested in blood, THC had the highest concentrations after vaping and THC-COOH had the highest concentrations after oral administration.

In urine, carboxy and hydroxy metabolites were present at higher concentrations than their parent cannabinoids. Peak cannabinoid concentrations were at 1 to 2 hours post vaped dosing and 3 to 4 hours post oral administration dosing. Peak metabolite concentrations were at 2 to 4 hours post vaped dosing and 4 to 6 hours post oral administration dosing. None of the targeted analytes in any biological sample correlated well with impairment measures for either route of administration. Detailed results for each targeted analyte are given below.

After vaping 20 mg of THC, peak average **THC** concentrations were approximately 40 ng/mL in blood (t_{max} = 0 hour), 1,000 ng/mL in oral fluid (t_{max} = 0 hour), and 12 ng/mL in urine (t_{max} = 1 hour). After oral administration of 25 mg of THC, peak average THC concentrations were approximately 3 ng/mL in blood (t_{max} = 2 hours), 125 ng/mL in oral fluid (t_{max} = 0 hour), and 5 ng/mL in urine (t_{max} = 4 hours).

After vaping 20 mg of THC, peak average **11-OH-THC** concentrations were approximately 1.5 ng/mL in blood (t_{max} = 0 hour), and 100 ng/mL in urine (t_{max} = 2 hours). After oral administration of 25 mg of THC, peak average 11-OH-THC concentrations were approximately 3 ng/mL in blood (t_{max} = 2 hours) and 150 ng/mL in urine (t_{max} = 4 hours).

After vaping 20 mg of THC, peak average **THC-COOH** concentrations were approximately 5 ng/mL in blood (t_{max} = 1 hour), and 40 ng/mL in urine (t_{max} = 4 hours). THC-COOH was detected in oral fluid of only one participant. Maximum concentrations for placebo and active vaped dosing sessions were less than 0.10 ng/mL. After oral administration of 25 mg of THC, peak average THC-COOH concentrations were approximately 15 ng/mL in blood (t_{max} = 4 hours) and 125 ng/mL in urine (t_{max} = 6 hours). THC-COOH was detected in oral fluid of only three participants. Two had maximum THC-COOH concentrations near 50 ng/mL and one had maximum THC-COOH concentration near 0.1 ng/mL.

After vaping 20 mg of THC peak average **CBD** concentrations were approximately 50 ng/mL in oral fluid (t_{max} = 0 hour), and 3 ng/mL in urine (t_{max} = 2 hours). In blood CBD was detected in only 5 active dosing timepoints across 5 participants with peak concentrations near 10 ng/mL (t_{max} = 0 hours). After oral administration of 25 mg of THC peak average CBD concentrations were approximately 1 ng/mL in urine (t_{max} = 3 hours). CBD was detected in blood from only one participant with a maximum concentration of 7 ng/mL and at low concentrations (1 ng/mL) in oral fluid from only 3 participants.

After vaping 20 mg of THC peak average **CBN** concentrations were approximately 2 ng/mL in blood (t_{max} = 0 hours), 75 ng/mL in oral fluid (t_{max} = 0 hours), and 1.5 ng/mL in urine (t_{max} = 1 hours). After oral administration of 25 mg of THC peak average THC concentrations were approximately 0.5 ng/mL in blood (t_{max} = 2 hours), 25 ng/mL in oral fluid (t_{max} = 0 hours), and 20 ng/mL in urine (t_{max} = 3 hours).

Delta8-THC was not detected in any urine samples; however, **delta8-THC-COOH** was detected at low concentrations in samples from 5 participants after the high dose oral administration (peak concentration 6 ng/mL at 5 hours). **8-OH-THC** was not detected in any

urine samples; however, **8,11-diOH-THC** was detected in samples from both routes of administration. After vaping 20 mg of THC, peak average 8,11-diOH-THC concentrations were approximately 10 ng/mL (t_{max} = 2 hours). After oral administration of 25 mg of THC peak average 8,11-diOH-THC concentrations were approximately 60 ng/mL in urine (t_{max} = 5 hours).

After vaping 20 mg of THC peak average **THCV** concentrations were approximately 1 ng/mL (t_{max} = 1 hour). THCV was not detected in urine samples after oral administration doses. After vaping 20 mg of THC peak average **THCV-COOH** concentrations were approximately 6 ng/mL in urine (t_{max} = 2 hours). After oral administration of 25 mg of THC peak average THCV-COOH concentrations were approximately 20 ng/mL in urine (t_{max} = 5 hours).

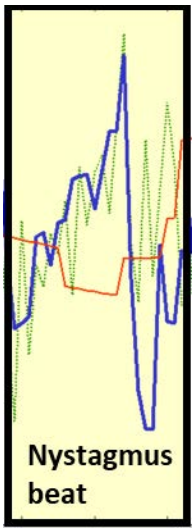
Progensis QI software was used to align and peak pick non-targeted data acquired from blood samples in positive and negative ionization modes. Data were compared by subject, dose, and route of administration to gather information on candidate ions that may be of interest for determining cannabis impairment. A potential target candidate list was generated consisting of approximately 4,500 individual components. Statistical analysis and correlation of these components to the subjective, cognitive, and psychomotor assessments administered were completed using a mixed effects model.

From these analyses, a narrowed potential target compound list was created consisting of the components with the five highest effect sizes for each assessment, components that were among the top 25 effect sizes for three or more assessments, and components that had a DRUID total impairment score effect size greater than positive 1. Based on these criteria 165 potential targets were included for oral administration and 152 potential targets were included for the vaporization administration. This subset of potential target compounds was prioritized for identification. Using m/z values and retention times the subset was searched against several

databases using the Progensis QI software. Between 50 and 75 potential targets across the doses and ionization modes had hits for tentative identifications that had M+H, M+Na, M+H-H_2O, M-H, or M-H_2O within 5 ppm of the observed m/z. This subset was then evaluated to determine if there were potential candidate compounds that should be investigated further. Several endocannabinoids and prostaglandins were among the tentative identifications made. Further investigation of some of the tentative identifications is needed such as reviewing fragmentation patterns and acquiring standards and checking their retention times to those of the extracted blood samples. An example of some of the molecular formulas of interest that lack a tentative identification in positive ionization are $C_{20}H_{30}O_2C_{18}H_{35}NO_2$ and $C_{20}H_{37}NO_2$. An example of a molecular formula identified in negative ionization is $C_{20}H_{34}O_5$. One component of interest is a compound with the same accurate mass as CBN that eluted prior to the reference standard retention time.

Eye Tracking

Algorithms were developed that successfully identified nystagmus and pupillary responses to light. For horizontal gaze nystagmus and convergence tests, the center of the pupil location was tracked via the x and y pixel location. Nystagmus was detected from the time series of the x-center coordinates. Processed eye tracking videos were interpolated to the original frame rate and filtered to highlight when nystagmus was present (blue trace) compared to absent (red trace). Filtering also served to distinguish nystagmus (blue trace) from noisy tracking (green trace) where peaks occur at a much higher frequency. Peaks were noted in the filtered signal, and only certain peaks above a threshold were retained. Nystagmus was detected when there were



This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

several peaks occurring within a short time frame. Automated nystagmus analysis agreed well with DRE determined nystagmus.

For delayed constriction and rebound dilation tests, the size of the pupil was estimated rather than the position. The approximate center was identified and a series of horizontal lines sweeps were then analyzed in the region of the estimated center. For each sweep, the beginning and end of the pupil are indicated by a large slope in pixel intensity values. The exact time of the light being turned on was determined based on the abrupt change in the mean intensity level of the entire image. The constriction time was computed as the time between the light being turned on and the time that the pupil diameter was within 2 pixels of the minimum size. Rebound dilation was indicated by an increase in pupil diameter of at least 10% after maximum constriction.

Scholarly Products

Grabenauer M, Vandrey R, Spindle T. "Differences in Cannabis Impairment due to Route of Administration"
Presented at Pittcon. March 17-21, 2019 Philadelphia, PA.

Martin E, Spindle T, Grabenauer M, Vandrey R "Assessment of Impairment Following Oral and Vaporized Cannabis Use in Infrequent Users: Preliminary Results."
Poster presented at Cannabis Science Conference. April 8, 2019, Baltimore MD.

Spindle T, Grabenauer M, Martin E, Vandrey R **"**Assessment of Impairment Following Oral and Vaporized Cannabis Administration in Infrequent Cannabis Users."
Presented at College on Problems of Drug Dependence (CPDD). June 15-19, 2019 San Antonio, TX.

Spindle, T "Cannabis Drug Testing and Measurement of Impairment."
Presented at the National Safety Council meeting on cannabis use in the workplace (titled "cannabis, its complicated"). June 26, 2019 Chicago, IL. Invited presentation.

Grabenauer M "Differences in Cannabis Impairment due to Route of Administration."
Presented at the Medical Review Officer Certification Committee (MROCC) R&D Symposium. June 28, 2019 Research Triangle Park, NC. Invited presentation.

Spindle T and Grabenauer M. "Assessment of Impairment Following Oral and Vaporized Cannabis Use in Infrequent Cannabis Users"

Presented at International Association of Chiefs of Police Annual training conference on Drugs and Impaired Driving (IACP-DAID). August 10-12, 2019 Anaheim, CA.

Grabenauer M, Vandrey R, Spindle, T "Detecting Cannabis Impairment after Cannabis Administration"
Presented at Society of Forensic Toxicologists (SOFT). October 13-18, 2019 San Antonio, TX

Spindle, T "Cannabis Drug Testing and Impairment: Evidence from Human Laboratory Studies." Presented at the Illinois Trucking Association (ITA) Summit on Cannabis. November 11, 2019 Chicago, IL. Invited presentation.

Elmore J, Spindle T, Grabenauer M, Vandrey R. "Assessment of Impairment Following Oral and Vaporized Cannabis Use in Infrequent Cannabis Users" Poster to be presented at Cannabis Science Conference, 2020, Baltimore MD. -postponed due to COVID-19, date TBD

Implication for Policy and Practice

The current understanding of pharmacokinetic and pharmacodynamic characteristics of cannabis administered via vaporization and oral consumption is limited. A greater understanding of these parameters will help determine whether or not an individual who has taken cannabis is impaired. Many jurisdictions with some form of cannabis legalization have enacted or are considering per se laws based on THC concentration in blood for cannabis impairment. Per se laws are advantageous for prosecution because they explicitly define an analyte and a cut-off concentration for that analyte; if a person has levels of that analyte above the cut-off concentration, that person is considered intoxicated and no further evidence of impairment need be demonstrated. Our work indicates that THC is not a reliable marker of cannabis impairment. Many participants had low levels of THC in their blood and oral fluid at timepoints where they exhibited substantially decreased performance on cognitive and psychomotor assessments. After oral administration at 10 mg THC only 2 participants reached a blood THC level greater than or equal to 5 ng/mL (1 max at 5 ng/mL, 1 max at 7 ng/mL). After oral administration at 25 mg THC only 6 participants reached a blood THC level greater than or equal to 5 ng/mL (2 max at 5 ng/mL, 3 max at 6 ng/mL, 1 max at 8 ng/mL).

Appendix

Abbreviations

THC – delta9-tetrahydrocannabinol

THC-COOH – delta9-tetrahydrocannabinol carboxylic acid metabolite

Delta8-THC – delta8-tetrahydrocannabinol

Delta8-THC-COOH – delta8-tetrahydrocannabinol carboxylic acid metabolite

THCV – tetrahydrocannabivarin

THCV-COOH – tetrahydrocannabivarin carboxylic acid metabolite

8-OH-THC – 8-hydroxy-tetrahydrocannabinol

11-OH-THC – 11-hydroxy-tetrahydrocannabinol

8,11-diOH-THC – 8,11-dihydroxy tetrahydrocannabinol

CBD – cannabidiol

CBN – cannabinol

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.