2022

Evaluating Cannabinoid Interference and Drug Stability in Oral Fluid for DUI/D Testing

Jasmine Maxwell
University of Alabama at Birmingham

EVALUATING CANNABINOID INTERFERENCE AND DRUG STABILITY IN ORAL FLUID FOR DUI/D TESTING

by

JASMINE MAXWELL

CURT E. HARPER, COMMITTEE CHAIR
ELIZABETH GARDNER
ROBERT LOCKWOOD

A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Master of Science

BIRMINGHAM, ALABAMA

2022

EVALUATING CANNABINOID INTERFERENCE AND DRUG STABILITY IN ORAL FLUID FOR DUI/D TESTING

JASMINE MAXWELL

FORENSIC SCIENCE

ABSTRACT

Oral fluid (OF) drug testing was first applied in the workplace and pain management facilities, and is now being applied to DUI/D (Driving Under the Influence of Drugs) cases to help establish probable cause and for laboratory evidentiary confirmation. OF drug testing is efficient due to its easy, fast, gender-neutral and OF sample collection is minimally invasive compared to blood and urine.

The objective of this study is to investigate the stability of cannabinoids in OF specimens over time. Limit of detection and potential matrix and analyte interference will also be evaluated.

The ANSI/ASB Standard 036 for Method Validation in Forensic Toxicology on how to evaluate interference and limit of detection was followed in this study. OF samples were collected from DUI/D subjects with a Quantisal collection device. Stability and interference studies were conducted using the ADFS OF cannabinoid assay. This method consists of a liquid-liquid extraction for delta-9-tetrahydrocannabinol (Δ^9-THC), delta-8-tetrahydrocannabinol (Δ^8-THC), 11-hydroxy-delta-9-THC (THC-OH), 11-nor-9-carboxy-delta-9-THC (THC-COOH), cannabidiol (CBD), cannabinol (CBN), and cannabigerol (CBG) for analysis by the Agilent 6460 or 6470 Triple Quadrupole Tandem Mass Spectrometer. Simulated and DUI case specimens were tested at time zero, two weeks, one month, 60 days, 90 days, two years, two and half years, and three years. The

novel cannabinoids Δ^8-THC and Δ^{10}-THC were validated by assessing limit of detection (LOD), analyte interference, and matrix interference.

Baseline resolution was achieved for Δ^8 and Δ^{10}-THC. An LOD was set at 1 ng/mL for both Δ^8 and Δ^{10}-THC. Δ^8, Δ^9, and Δ^{10}-THC were not stable when stored at room temperature. In fact, Δ^{10}-THC was no longer detected at 60 days. Overall, target stability was greatly enhanced with refrigeration (4°C). Δ^9-THC was stable for up to 90 days, with overall target stability within ±20% of the concentration at time zero. Previously analyzed case samples had a median decrease of 20% when compared to the first analysis, falling within ±20% of the initial concentration. The elution buffer, collection device, analyte chemical properties, and storage conditions are all factors in the stability of drug concentrations in OF.

These results illustrate the importance of continuously monitoring method performance, potential new interferents, and analyte stability of cannabinoids.

Keywords: Cannabinoid Stability, Interference, Oral Fluid, Driving Under the Influence of Drugs, Quantisal, Alabama Department of Forensic Science

ACKNOWLEDGMENTS

Foremost, I would like to express my sincerest gratitude to my advisor and mentor, Dr. Curt E. Harper, for the continuous support throughout my thesis research. Without his guidance and persistent help this thesis would not have been possible.

I would like to thank my committee members, Dr. Elizabeth Gardner and Dr. Robert Lockwood, for their encouragement, insightful comments, and hard questions. My sincerest thanks goes to Kristin Tidwell for her mentorship and willingness to help throughout my research. I would also like to recognize all the scientists of the Alabama Department of Forensic Sciences Toxicology Discipline for their willingness to assist with anything I needed help with rather it be troubleshooting an instrument or learning a new extraction technique on top of their day-to-day task.

In addition, a thank you to Cody Paseur for assisting with the LOD studies. I also thank Cindy Coulter and Dani Mata for their willingness to answer questions in regard to their previous cannabinoid research. Lastly, I am thankful for the Alabama Department of Forensic Sciences for allowing me to do my research at their laboratory.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADFS	Alabama Department of Forensic Sciences
DRE	Drug Recognition Expert
DUI/D	Driving Under the Influence of Drugs
LC/MS	Liquid Chromatography Mass Spectrometry
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
MPA	Mobile Phase A (organic)
MPB	Mobile Phase B (aqueous)
Rs	Roadside screen
OF	Oral fluid
OFFS	Oral fluid field screening
S/N	Signal-to-Noise
SFST	Standardized Field Sobriety Testing
SOP	Standard Operating Procedure
TIC	Total Ion Chromatogram
ULOQ	Upper Limit of Quantitation

INTRODUCTION

Applications of OF Testing

Testing oral fluid (OF) and saliva for drugs of abuse has been studied since the 1990's.[1] In Europe, OF testing in driving under the influence of drugs (DUI/D) cases began in 2010.[2] OF consists predominantly of water, proteins, electrolytes, mucin, enzymes, epithelial cells, cholesterol, and vitamins, which comes from a combination of gingival crevicular fluid, and fluid from salivary parotid, submandibular, and sublingual glands.[3] Saliva is the fluid collected from the parotid gland, and is free from mucosal cells and food residues.[4] Lipid-solubility, pH of the matrices, and pKa of the drug are some of the physicochemical factors that affect the transfer of drug molecules from blood to saliva. For example, plasma pH is regulated at 7.4 but the pH of saliva can fluctuate significantly due to an increase in rates of secretion caused by smell, taste, pain, chewing, and medications.[5]

OF testing in the workplace can help deter employees from using drugs or alcohol. The sample is easy to collect and contains the pharmacologically active drug form. Drugs in an OF sample typical indicate recent drug use when an appropriate window of detection and cutoff is selected.[6] Pain management facilities commonly analyze urine and OF in clinical compliance monitoring.[7] An advantage of OF testing at pain management facilities is the ease of sample collection. It also provides confidence that the sample has not been adulterated. Medical staff at pain management facilities prefer OF collection as it provides a higher number of positive results than urine for all analytes including illicit drugs.[8]

There are two distinct types of OF testing relative to DUI investigations. Oral Fluid Field Screening (OFFS), also known as roadside screening (Rs), and evidentiary confirmation testing by the laboratory. Law enforcement utilizes OFFS devices to establish probable cause in a number of states including Alabama, Arizona, Michigan, and Indiana. OFFS devices, such as the Draeger DT5000 and Abbott SoToxa, are used by law enforcement to obtain a preliminary result for possible drug use.[9] Lastly, an emerging use of OF is for assisting medical examiners and coroners in establishing manner and cause of death, especially in suspected overdoses. Compared with clinical specimens, postmortem specimens are often more difficult to analyze for drugs of abuse due to matrix challenges. OF provides medical examiners and coroners an alternative matrix for toxicological analysis.[10]

OF testing is gaining acceptance due in part to efforts of the Society of Forensic Toxicologists Oral Fluid Committee, which has provided guidelines for OF pilot projects in forensic toxicology. The guidelines provide an outline of the pilot projects that laboratories and law enforcement agencies may go through to establish an OF program in their state or jurisdiction. The Committee's mission is to provide scientifically based information and resources to toxicologists, law enforcements, prosecutors, and the general public regarding the utility of OF analysis.[11] The SOFT/AAFS Oral Fluid Committee sent out their third annual survey on Oral Fluid Drug Testing on November 24th, 2020. The survey was sent to 86 toxicologists throughout the United States and one lab in Canada. The survey was sent through Survey Monkey and included 25 questions inquiring about the status of OF programs in the United States (Appendix A). The survey had a 71% response rate. Many of the questions in the SOFT/AAFS Oral Fluid

Committee third annual survey were directed towards OF testing in a respondent's prospective lab as well as OF testing in his/her state.[12] While 95% of respondents said their laboratory performs DUI/D testing, 51% of respondents did not have a state statute that allowed for OF drug testing in DUI/D cases. Vermont, Kansas, and Oklahoma passed bills to allow for OF drug testing. New York and Ohio are currently in progress of developing and/or validating OF drug evidentiary testing. Wisconsin has validated OF drug evidentiary testing but are pending a statute change to allow for OF testing. Alabama is the only state that is currently testing OF for evidentiary purposes at the laboratory.

Advantages of OF Testing

OFFS screening is minimally invasive and provides rapid results (i.e. within 10 minutes) at the scene of the traffic stop or crash. OF screening can be used with other evidence, such as Standardized Field Sobriety Test (SFSTs), to build probable cause for arrest decisions. Screen results can also identify potential polydrug impaired drivers regardless of their blood alcohol concentration (BAC) level and support search warrant requests for additional biological samples.

In addition to ease of collection, OF testing has other advantages relative to blood or urine testing. In DUI/D cases, the collection of OF occurs close to the time of driving which allows for detection of pharmacologically active or impairing drugs. Samples that contain basic drugs (pka 8-12[13]) will concentrate in OF in comparison to blood. As the pH decreases in OF, a greater portion of drug will be ionized which in turn increases the drug

concentration in OF.[14,15] OF is also less expensive to collect than blood due to the lack of medical personnel needed in the collection process.

Blood and OF share similar windows of detection for drugs; but their concentrations may not be equivalent. Window of detection is the time that the drug can be detected in a biological sample above a specified instrument cut-off. Higher concentrations are typically observed in OF compared to blood due to the routes of administration of the drug, e.g orally, smoke, or insufflated. High OF drug concentrations are often detected after recent use due to oral cavity coating or contribution. If a drug is injected, an oral coating will not be present, but drug will still be present due to partitioning between the blood and OF. Urine is an inferior matrix to blood and OF as it provides less information regarding recent past drug use or exposure.

Advantages of OF testing in DUI/D Cases

The use of OF testing with a OFFS device is an additional tool for law enforcement. The OFFS devices, Draeger DT5000 and Abbott SoToxa, test for methamphetamines, amphetamines, opioids, cocaine, marijuana, and benzodiazepines. These devices should only be used to establish probable cause. Figure 1 contains a workflow schematic adapted from the AAA Infographic for the collection of samples during a DUI stop.[16]

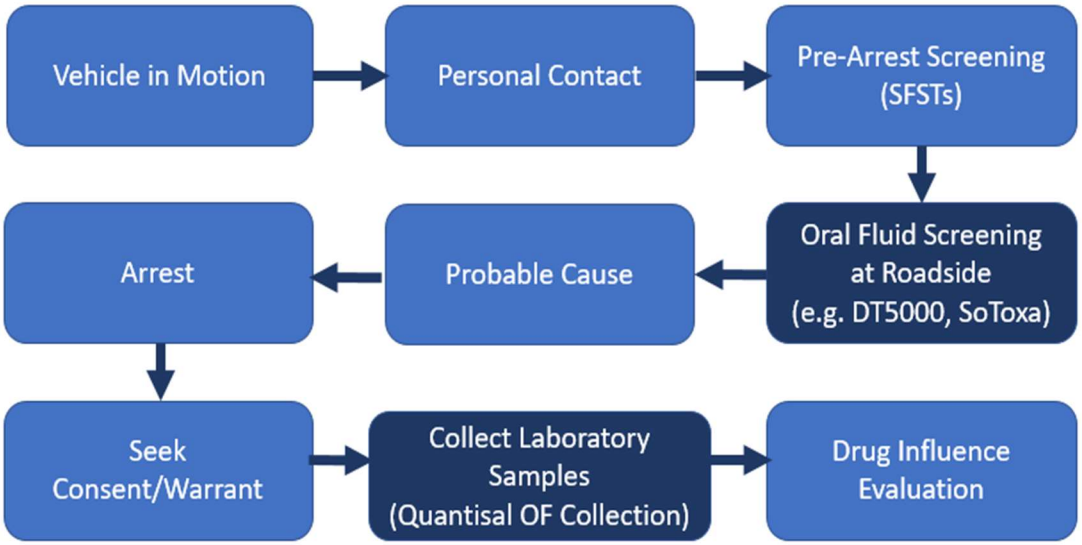

Figure 1: AAA Infographic detailing proper time points to collect OF during an Investigation[15]

Results from an OFFS provide a preliminary report of the drugs in a suspect's system. This single use test is conducted by law enforcement and does not undergo further testing by the laboratory. The OF screening results may be admissible in hearings for probable cause in some jurisdictions. A second sample is collected using a collection device such as the Quantisal at the roadside allows the officer to collect an OF evidentiary, confirmation specimen for laboratory testing. Officers do not need a medical professional to draw the sample, which eliminates transport time and cost. Lastly, the officer does not have to be of the same sex to collect the sample.

Limitations of OF Testing

A common limitation with OF testing is that subjects can have reduced salivation. Salivation decreases after stimulant, opioid, and marijuana use, potentially extending the required time for obtaining an adequate specimen volume.[17] Drugs that are neutral, amphoteric, or acidic, such as benzodiazepines and opiates, do not partition well into OF

due to the process of ion trapping. Ion trapping occurs when an ionized drug is trapped on one side of the cell membrane that divides two compartments with fluids of different pH values. This creates challenges for detection even with supra-therapeutic use with doses that are greater than for therapeutic use. Dry mouth is another issue common in users of stimulants and marijuana due to route of administration by smoking that results in little to no salivary stimulation. Total OF-elution buffer volume is typically low in this scenario, restricting the number of confirmatory tests that can be performed by the laboratory. For example, Quantisal collection device, for evidentiary testing, collects one milliliter of OF that is extracted into three milliliters of buffer, resulting in a total volume of approximately four milliliters. OFFS devices typically use all the sample provided and are not intended to be sent to the laboratory for evidentiary testing in most programs. Another sample must be required to perform confirmatory testing; therefore, the same sample cannot be used for both screening and confirmation. Finally, OF testing is not common in most forensic laboratories and would require method development and validation to be completed.[4] At this time only Alabama, Wisconsin, and California have developed confirmation methods, while Alabama is the only state crime laboratory currently offering OF confirmatory testing.

ADFS OF Drug Testing DUI/D Program

At this time, the Alabama Department of Forensic Sciences (ADFS) has the only comprehensive OF DUI testing program in the country with confirmation, evidentiary testing at the laboratory. Once the OF and/or blood samples are collected by an officer, the samples are sent to ADFS. ADFS offers evidentiary confirmation, following post-

arrest (Figure 1), and tests samples for 25 drugs of abuse and therapeutic drugs commonly found in DUI/D cases. These results can be used as evidence in court proceedings.

Alabama is the first state to offer both approved OFFS devices and in-house evidentiary confirmation testing for OF. The first OF case was submitted to the laboratory in August 2018.[18] Prior to implementing OF testing, the ADFS conducted an OF pilot project which resulted in the validation of in-house evidentiary confirmation testing and three approved OFFS devices. The stakeholders for the project included Drug Recognition Experts (DRE), toxicologists, laboratory directors, and district/municipal attorneys.

The laboratory compared the roadside or OF results to the confirmation.[19] Three roadside OF screening devices were validated and approved for use during a DUI/D stop or accident to establish probable cause: the Draeger DT5000, Abbott SoToxa, and Randox Multistat. The Quantisal collection device was approved for evidentiary, confirmation OF samples. The Quantisal contains a collection pad made of absorptive cellulose and polyethylene, a polypropylene stem attached to the pad, and a plastic transport tube containing contain three milliliters of buffer. To collect samples using Quantisal, the collection device pad is placed under the subject's tongue until enough volume is collected and the indicator turns blue. It is important to ensure the subject has not eaten or drunk anything within the last 10 minutes to eliminate possible interferents. The applicator, containing one milliliter of the collected OF, is placed back into the Quantisal tube with three milliliter of blue liquid containing a buffer that assists in extracting the drugs from the collection pad and that stabilizes the drugs present in the

sample until it can be tested.[20] Once the specimen is received at ADFS, the buffer solution containing the sample is transferred into a plastic screw cap tube. The laboratory analyzes the sample and reports the drugs present in a Toxicological Analysis Report. In the biological specimen kits provided to officers for roadside DUID testing, there is an OF collection device and two blood collection tubes. It is considered best practice to collect both blood and OF. In most circumstances, testing both specimens will provide a more complete picture of recent drug use.[21]

Alabama has an implied consent statement used in DUI investigations that states that any person who operates a vehicle within the state is deemed to have consented to a blood, breath, or urine test to measure blood alcohol content (BAC) only if lawfully arrested for driving under the influence (DUI). A recent update of Alabama's implied consent bill, SB 258, went into effect on August 1st, 2021. The update expands the implied consent provision for offenders from just alcohol to "any impairing substance", while allowing OF to be collected as an evidentiary sample. It also expanded the revocation of driving privileges for refusals.

Novel Cannabinoid Interference in OF

In 2021, Δ^9-THC was the most prevalent drug present in DUI cases in Alabama with a 45% positivity rate. Ethanol was the most prevalent drug in traffic fatalities followed by Δ^9-THC (38%). Δ^9-THC, the psychoactive compound in marijuana, has a stronger correlation to recent use in OF than in blood. Δ^9-THC is metabolized to Δ^9-THC-OH, which is an intermediate active metabolite. THC-OH is then oxidized to Δ^9-THC-

COOH, which is inactive. Studies have shown that after an hour of ingestion subjects were positive for Δ^9-THC in OF.[22]

In 2018, the Agriculture Improvement Act, also known as the Farm Bill, legalized hemp and its derivatives at the federal level. The law defines hemp as, "the plant *Cannabis Sativa L*. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers whether growing or not, with Δ^9-THC concentration of not more than $< 0.3\%$ on a dry weight basis".[23] In late 2020, a final rule was made that clarified that tetrahydrocannabinols do not include any compound derived from hemp. The final rule also stated that "marijuana extract" is limited to any formulation containing > 0.3% THC. This allowed hemp-derived cannabinoids such as Δ^8-THC, Δ^{10}-THC, THC-O, and THC-P.

Δ^8-THC is not produced by the plant but synthetically derived from CBD. The pharmacology of Δ8-THC and Δ^9-THC both interact with the endocannabinoid system, specifically the CB1 and CB2 receptors. However, Δ^8-THC has less affinity for the cannabinoid receptors resulting in a lower potency. The potency of Δ^8-THC is estimated at 50% to 66% less than that of Δ^9-THC.[24]

Δ^8, Δ^9 and Δ^{10}-THC differ only in the location of a double bond. The major difference new users have experienced is that Δ^{10}-THC is similar to cannabinoids from the sativa strain, known for giving users a more energizing high. Δ^{10}-THC was discovered accidentally when hemp was accidentally exposed to fire-retardant spray, which produced crystals of the Δ^9-THC analog. Similar to Δ^8-THC, Δ^{10}-THC is a synthetic cannabinoid due to the fact that the compound is not produced by the plant.[25]

Unlike Δ^8-THC, THC-O and THC-P fall into a “gray area” due to due to their strictly synthetic status. THC-O, also known as THC-O acetate, is a synthetic form of THC originally created by the Army Chemical Corps as a non-lethal incapacitating agent tested on dogs.[26] It is a precursor to Δ^8-THC. THC-O takes approximately one hour to produce effects that are around three times more potent than Δ^9-THC. Likewise, THC-P is said to be 30x as potent as Δ^9-THC due to its natural seven-carbon acetyl chain.[27] Cannabinoids like Δ8, Δ9, and THC-P bind to CB1 receptor producing psychoactive effects. However, THC-P binds to the receptor for a longer period, making the effects stronger. Δ^9-THC legal status has changed over recent years in many states. Figure 2 shows a map adapted from the American Nonsmokers' Rights Foundation website that displays which states have legalized recreational and medical marijuana, medical only marijuana, and where marijuana is illegal.

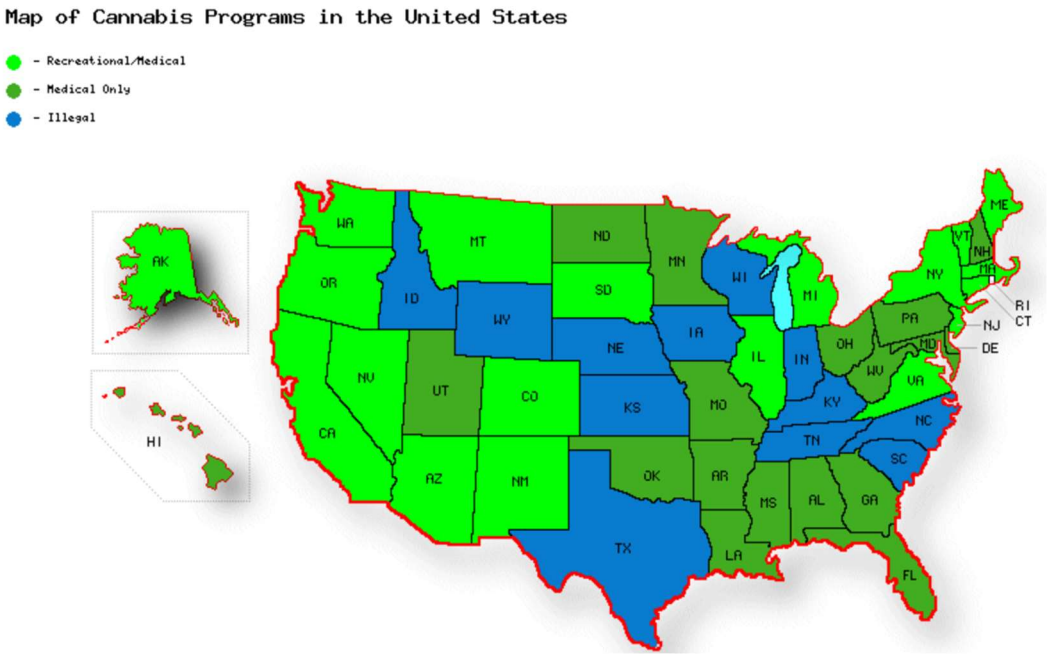

Figure 2: Map of Cannabis Programs In the United States[28]

In states such as Colorado, where Δ^9-THC is legal, both recreationally or medically, Δ^8-THC is banned. A map displaying the current legal status of Δ^8-THC in the United States adapted from CBD oracle is shown in Figure 3.

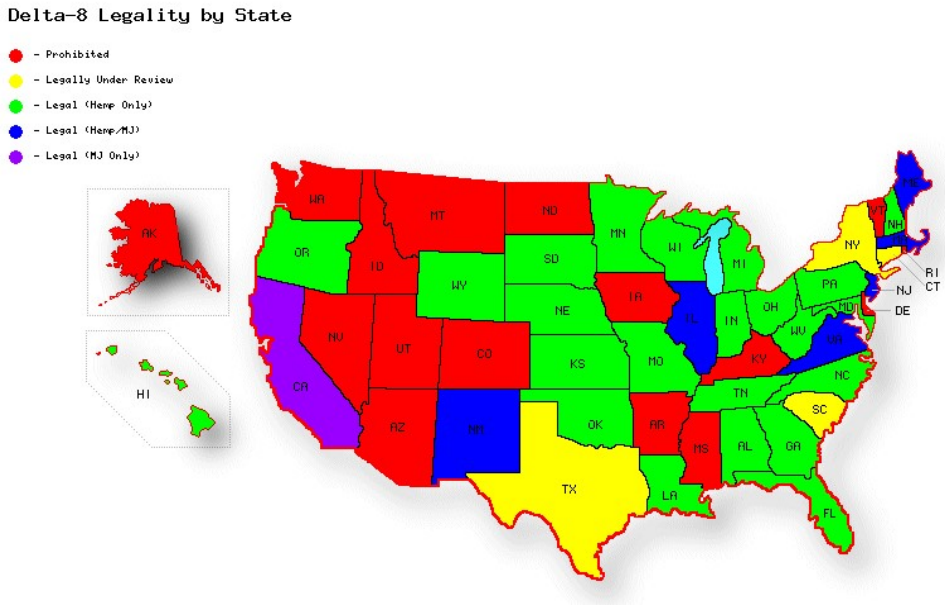

Figure 3: Δ^8-THC Legality in the United States[29]

While over 50% of the states allow Δ^8-THC, some states such as Alabama, Illinois, Oklahoma, and Oregon are currently reviewing the legal status as Δ^8-THC produces the same symptoms of impairment similar to Δ^9-THC.

Addition of New Targets

It is important that laboratories continuously monitor method performance and evaluate potential new interferents as these new cannabinoids could be misidentified due to the fact that several compounds are isomers of Δ^9-THC. Many of these new novel cannabinoids share the same chemical formula as Δ^9-THC but the arrangement of atoms is what differentiates them from one another, making them isomers of Δ^9-THC. As new

novel cannabinoids have become more popular, it is important to update the laboratory's methods to include these novel compounds. The ANSI/ASB 036 Standard provides the parameters for method validation and material modifications. It is important when adding a target to an existing method to determine if the new target produces any interference with previously validated targets and to determine the limit of detection (LOD) for the new compound.

An LOD is the lowest concentration that can yield a reproducible response greater than three times the noise level of the background signal from negative samples.[30] The target compound also has to fall into the accepted criteria for retention time, signal to noise (S/N), and peak shape. LOD studies can be performed in multiple ways, but the most common when adding a new target to a method is to estimate the LOD by using reference standards. For example, blank matrix can be fortified at decreasing concentrations of the target analyte in duplicate over three days.

Co-elution and interference from other commonly encountered analytes, such as Δ^8 and Δ^{10}-THC in this case, should be evaluated for potential interference with the method's validated targets. Studies are performed by analyzing fortified matrix samples, previously analyzed samples, or neat reference materials for potential interferences. The most common drugs encountered should be included in the evaluation together with other common drugs in the same drug category. This could include creating mixtures of validated targets with potential interference analytes to determine if interferences are occurring with validated targets (e.g. Δ^9-THC).

When co-elution of compounds occurs in methods for liquid chromatography tandem mass spectrometry (LC/MS/MS), an improvement in the chromatography is

needed. There are many ways of improving chromatography. Some modifications that can be made in the method can be changing the mobile phase (e.g., gradient, composition), changing the stationary phase, optimizing the column efficiency (e.g., size, dimension, pore size), and altering the temperature of the column. Gradient modifications are normally the simplest modification. The gradient affects the chemical separation and elution. Typically, a gradient will begin with a "weak" elution and end with a "strong" elution condition. Weaker elution conditions are used so compounds will not immediately elute from the chromatography column. Linear gradients are typically used in labs as compounds will stick to the column until the solvent polarity is strong enough to overcome their interactions with the stationary phase and elute off the column.[31]

Stability of Cannabinoids

Stability of drugs is a common parameter tested when validating toxicology methods. The stability of drugs in OF depends upon the type of collection device, elution buffer, and how the samples are stored.[32] For example, acidic buffers were found to help with the delay or prevention of degradation of more chemically instable compounds such as Δ^9-THC or 6-monoacetylmorphine.

The stability of drugs during storage and transportation of the Quantisal collection devices has been examined. Immunalysis (2017), the company that produces the Quantisal collection device, investigated analyte stability with samples stored at room temperature and refrigerated. Samples were examined on the day of collection and seven, 14, and 30 days post-collection.[33] Samples were analyzed in duplicate at each time point and storage condition. Drugs with higher concentrations demonstrated extended stability.

Samples stored at room temperature remained stable for 30 days with the exception of Δ^9-THC, which showed significant loss after seven days. However, the samples stored in refrigerated conditions showed all drugs to be stable for at least 30 days with only 2-10% decrease in analyte concentration.

Moore (2006) also examined the percentage of Δ^9-THC that can be recovered from the collection pad. [34] To determine recovery from the pads, samples were spiked at 3, 5, and 6 ng/mL using neat OF fortified with Δ^9-THC. Collector pads from Quantisal were dipped into the expectorate fortified solution until the indicator on the collector swab turned blue. Swabs were then placed into transportation tubes containing Quantisal buffers and left at room temperature overnight. Results showed that lower target concentrations resulted in greater recovery of the target analyte. Samples were stable in the Quantisal collection device with less than 10% degradation from the original concentration.

Crouch (2005) compared the Quantisal collection device and the Intercept device. Intercept collection device samples stored at 4°C had greater than a 50% decrease in drug concentration from time-zero to two weeks.[35] The Quantisal collection device saw improved Δ^9-THC stability than the Intercept collection device with no significant loss Δ9-THC after two weeks. Stimulation, pH, the volume of OF collected and recovered, drug recovery and stability were considered as limitation factors in this study. This study proved that the Quantisal collection device allows for improved stability of Δ^9-THC at 4 °C compared to the Intercept collection device.

Another aspect of stability examined was the effect of lipophilic mouth cells on Δ^9-THC stability. It was hypothesized that low numbers of mouth cells, when collected,

can impact the quantified Δ^9-THC concentrations in OF during centrifugation. A comparison of the Quantisal collection device and the Certus collector found that the Certus collector required more mechanical movement which resulted in the collection of more mouth cells. The results showed that without the presence of mouth cells, Δ^9-THC concentrations decreased in both the Quantisal and Certus collectors at day seven compared to day 1and increased significantly at day 14.[36]

OBJECTIVES/AIMS

1. To evaluate novel cannabinoid interference in OF cannabinoid assay
    a. Evaluate matrix interference (e.g. expectorant, Quantisal, Oral-Eze)
    b. Evaluate novel cannabinoid interference (Δ^8-THC, Δ^8-carboxy-THC, Δ^8-hydroxy-THC, 9R-Δ^{10}-THC, 9S-Δ^{10}-THC, 9R-$\Delta^{6a,10a}$-THC, 9S-$\Delta^{6a,10a}$-THC, THC-O, THC-P)
2. To validate Δ^8-THC and Δ^{10}-THC and add to existing OF cannabinoid assay
    a. Evaluate limit of detection for Δ^8-THC and Δ^{10}-THC
    b. Evaluate analyte and matrix interference
    c. Evaluate carryover

3. To evaluate stability in simulated case work specimens in different storage conditions over various time points

    a. Simulated casework at time-zero, two weeks, 60 days, and 90 days at different storage conditions: 20-22°C, 4°C, and -20°C

4. To evaluate stability in previously analyzed case specimens stored at 4°C over various time points

    a. Re-analysis of previously analyzed casework at 2.5 years

    b. Evaluate average time between shipment and receive, first analysis and receive, and overall process

MATERIALS AND METHODS

Materials

Standards Used for Calibrators, Control, and Interference Evaluation

Table 1: List of Standards Used for OF Cannabinoid Assay & Interference Evaluation

Target	Manufacturer	Manufacturer location
Δ^9-THC-D3	Cerilliant	Round Rock,TX
Δ^9-THC-OH-D3	Cerilliant	Round Rock,TX
Δ^9-THC-COOH-D3	Cerilliant	Round Rock,TX
Cannabidiol-D3	Ceriliiant	Round Rock,TX
Δ^9-THC	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Δ^9-THC-OH	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Δ^9-THC-COOH	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Cannabinol	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Cannabidiol	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Cannabigerol	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Δ^8-THC	Cerilliant & Lipomed	Round Rock,TX & Cambridge, MA
Δ^8-THC-OH	Cayman	Ann Arbor, MI
Δ^8-THC-COOH	Cayman	Ann Arbor, MI
9R-Δ^{10}-THC	Cayman	Ann Arbor, MI
9S-Δ^{10}-THC	Cayman	Ann Arbor, MI
9R-$\Delta^{6a,10a}$-THC	Cayman	Ann Arbor, MI
9S-$\Delta^{6a,10a}$-THC	Cayman	Ann Arbor, MI
Δ^8-THC-O	Cayman	Ann Arbor, MI
Δ^8-THC-P	Cayman	Ann Arbor, MI
Δ^9-THC-O	Cayman	Ann Arbor, MI
Δ^9-THC-P	Cayman	Ann Arbor, MI

Table 1 above lists all standards from Cerilliant (Roundrock,TX), Lipomed (Cambridge, MA), and Cayman (Ann Arbor, MI). For the calibrator and controls were prepared using two different manufacturers as outlined in the standard operation procedure (SOP) for the ADFS OF Cannabinoid Assay. The calibrators were made using the Cerilliant non-deuterated standards and the controls were made using the Lipomed non-deuterated standards (Table 1). Standard concentrations from the manufacturer for all cannabinoids in Table 1 were 1.0 mg/mL. Stock solutions were made by diluting the standard with methanol to create stock solutions at concentrations of 0.01, 0.1, and 1 µg/mL. With each extraction, a calibration curve was analyzed using concentrations of 1, 2, 4, 10, 20, 40, 100, 200, and 300 ng/mL of delta-9-tetrahydrocannabinol (Δ^9-THC), delta-8-

tetrahydrocannabinol (Δ^8-THC), 9R and 9S delta-10-tetrahydrocannabinol (9R-Δ^{10}-THC, 9S-Δ^{10}-THC), 11-hydroxy-delta-9-THC (THC-OH), 11-nor-9-carboxy-delta-9-THC (THC-COOH), cannabidiol (CBD), cannabinol (CBN), and cannabigerol (CBG) .Controls were made and analyzed at the concentrations of 10, 40, and 100 ng/mL with the same targets mentioned above.

Methods

OF Cannabinoid by LC/MS/MS Procedure

The SOP of the ADFS laboratory for the analysis of cannabinoids in OF was followed in this project. This method consists of a liquid-liquid extraction that quantitates Δ^9-THC and metabolites, CBD, CBN, CBG and qualitatively identifies Δ^8-THC and Δ^{10}-THC isomers. The sample preparation for OF Cannabinoids assay is illustrated by flowchart in Figure 4.[37]

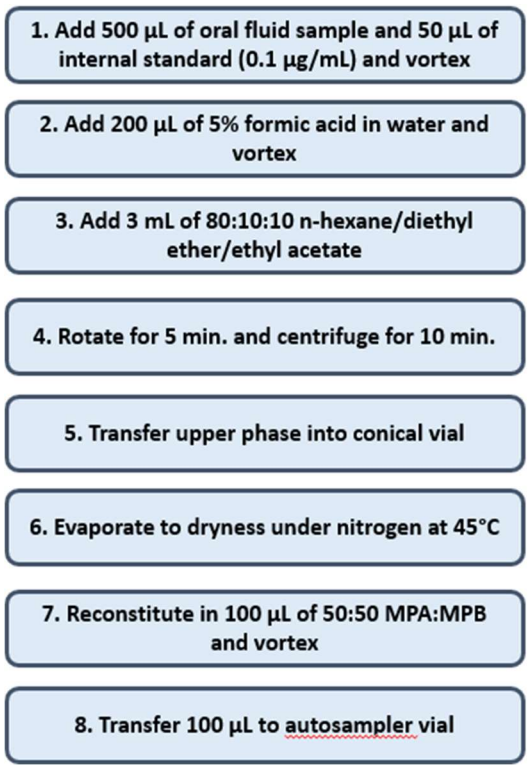

Figure 4: ADFS OF Cannabinoid Assay Sample Preparation Steps

20 µL of sample were injected onto the Agilent 6460 or 6470 Liquid Chromatograph/Triple Quad Mass Spectrometer (LC/MS/MS) with an Agilent 120 EC-C18, 2.1x100mm, 2.7 µm reverse phase column. Mobile phase A (MPA) was 0.1% formic acid in methanol and mobile phase B (MPB) was 5mM ammonium formate, 0.1% formic acid in water. The run time was 10 minutes with a two-minute post run to ensure all compounds had eluted off the column before the next 20 µL sample was injected. The final LC gradient for OF Cannabinoid method is shown in Table 2. All samples in this study were analyzed using this method.

Table 2: LC Gradient for Cannabinoids in OF

Time (min)	0.1% Formic Acid in Water (MPA%)	0.1% Formic Acid in Methanol (MPB%)	Flow (mL/min)
1.0 min	30	70	0.50
4.0 min	20	80	0.50
10 min	1	99	0.50
11 min	1	99	0.50

Method for Evaluating Interference of Novel Cannabinoids

Blank OF matrix samples were spiked with 11 different cannabinoids at 40 ng/ml. Twenty-nine synthetic cannabinoids were split between two stock solutions designated as Tier 1 and Tier 2 (Appendix C). Table 3 is a list of all targets and concentrations analyzed for interference.

Table 3: List of Cannabinoids Evaluated with Final Concentration (ng/mL)

	Target Evaluated	Final Concentration (ng/mL)
1	(6aR,9S)-Δ^{10}-THC	40
2	(6aR,9R)-Δ^{10}-THC	40
3	9R-$\Delta^{6a,10a}$-THC	40
4	9S-$\Delta^{6a,10a}$-THC	40
5	Tier 1 Synthetic Cannabinoids	40
6	Tier 2 Synthetic Cannabinoids	40
7	Δ8-THC	40
8	Δ8-THC-COOH	40
9	Δ8-THC-OH	40
10	THC-O	40
11	THC-P	40

LOD Validation to Add Δ^8-THC and Δ^{10}-THC to the OF Cannabinoid by LC/MS/MS Procedure

Immunalysis Negative Synthetic OF was spiked with Δ^8 and Δ^{10}-THC at concentrations of 1, 2, and 4 ng/mL in duplicate for three separate days. Carryover was assessed by running a blank after the highest calibrator as well as by analyzing blanks between case specimens. Robustness was evaluated by having another scientist repeat the extraction.

Storage Temperature Stability Study

A 1000 ng/mL stock solution of validated targets (listed in the OF procedure section above) as well as Δ^8 and Δ^{10}-THC isomers were prepared. Fourteen expectorate samples, each composed of 20 µL of expectorate were collected from each volunteer. Once expectorate samples were collected, they were centrifuged to remove any solid materials so only neat OF was left. Once centrifuged, 2 mL of expectorate OF was placed into a 16x100mm glass culture tube.

The first six samples of expectorate OF were spiked with 400 µL of the calibrator stock solution (100 ng/mL). The second set of six samples were made by adding 120 µL of the stock solution (25 ng/ml). The concentration of the standards was chosen in order to account for the four-fold dilution of the expectorant when extracted with the Quantisal collection device. Once spiked, samples were covered with aluminum foil to prevent light exposure. Samples were then placed on the rack rotator at 37 rpm for 2 hours and then the Quantisal collector swab was placed into the 16x100 mm tube to collect the spiked OF. Once the indicator turned blue the collector was placed into the collection tube containing

Quantisal buffer. The samples were kept for three hours at room temperature to allow proper drug absorption from the pad to the buffer. After three hours, the OF cannabinoid extraction (Figure 4) was performed to establish the initial or time zero (T0) concentrations. After T0 concentrations were established (50 and 15 ng/mL) the remaining samples were stored in their perspective storage conditions of 20 °C, 4 °C, and -20 °C. Table 4 shows the number of replicates per concentration and storage condition.

Table 4: Number of Samples per Storage Condition per Concentration

	Storage condition		
Target Concentration	20°C (n= # of replicates)	4°C (n= # of replicates)	-20°C (n= # of replicates)
25 ng/mL	2	2	2
100 ng/mL	2	2	2

Samples were removed from storage conditions at 14 days, one month, 60 days, and 90 days for analysis. Stability of the targets were calculated using the equation listed below, with c_1 being the new concentration and c_2 being the initial concentration.

$$\left[\frac{[c_1 - c_2]}{c_2}\right] x100$$

Casework Stability Study

Previously analyzed DUI cases that had met their two-year disposal date were analyzed using the Cannabinoid OF procedure. A list of the samples as well as first analysis results, the date they would reach 2.5 years was entered in a Microsoft Excel worksheet. The cases were reanalyzed when the 2.5 year time point was met, and the percent difference was calculated using the formula listed above. Previously analyzed OF cases were collected using the ADFS Quantisal collection protocol found in Appendix B.

Casework- Investigation of Time Between Collection, Shipment, and Analysis

A Microsoft Excel sheet was created to track the days between collection and shipment, between shipment and receipt at ADFS, between collection and first analysis, and between receipt and first analysis for the most recent 100 OF DUI cases submitted to ADFS. Results were sorted by date and the average, median, maximum, and minimum were calculated.

RESULTS

Interference with Novel Cannabinoids in OF

The interference study was conducted to determine if the non-validated cannabinoid standards and synthetic cannabinoids would interfere with the validated cannabinoids using the SOP for OF Cannabinoid by LC/MS/MS method. Table 5 below lists the targets analyzed and if interference occurred with validated targets.

Table 5: Preliminary Interference (Pre-Modification)

Potential Interferents	Pre-Modification Interference Occurred (Yes/No)	Target?
Δ^9-THC	Yes	Δ^8-THC*, (6aR,9s)-Δ^{10}-THC*, (6aR,9R)-Δ^{10}-THC*, 9R - $\Delta^{6a,10a}$ – THC*, 9S - $\Delta^{6a,10a}$ – THC*
Δ^8-THC	Yes	Δ^9-THC*, (6aR,9s)-Δ^{10}-THC, (6aR,9R)-Δ^{10}-THC, 9R - $\Delta^{6a,10a}$ – THC, 9S - $\Delta^{6a,10a}$ – THC
(6aR,9s)-Δ^{10}-THC	Yes	Δ^8-THC, Δ^9-THC*
(6aR,9R)-Δ^{10}-THC	Yes	Δ^8-THC, Δ^9-THC*
9R - $\Delta^{6a,10a}$ - THC	Yes	Δ^8-THC, Δ^9-THC*
9S - $\Delta^{6a,10a}$ - THC	Yes	Δ^8-THC, Δ^9-THC*
Tier 1 Synthetic Cannabinoids	No	
Tier 2 Synthetic Cannabinoids	No	

*Resolved, but without baseline resolution

Figure 5 shows the chromatogram of the targets interfering with Δ^9-THC. The first peak shown is Δ^9-THC which is not fully resolved from Δ^8-THC, isomers of Δ^{10}-THC, and isomers $\Delta^{6a,10a}$-THC. Furthermore, Δ^8-THC, isomers of Δ^{10}-THC, and isomers of $\Delta^{6a,10a}$-THC co-eluted. These findings warranted method adjustment.

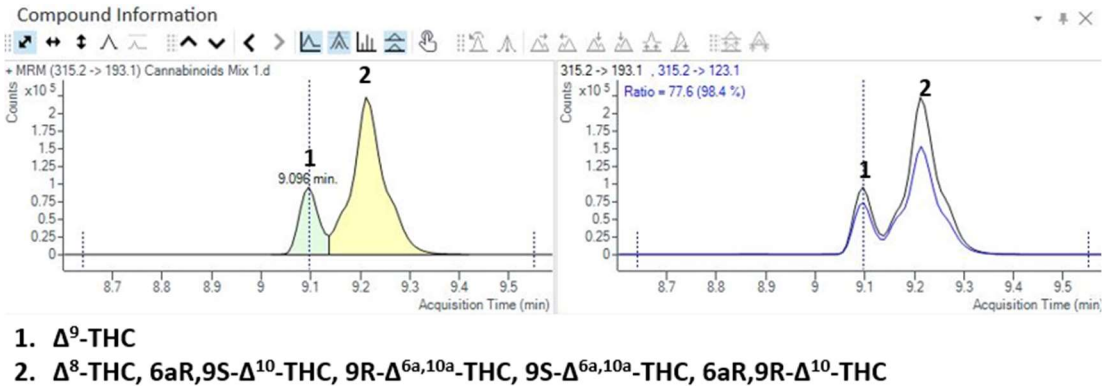

Figure 5: Preliminary Interference Run on OF Cannabinoid Gradient on 6460 Liquid Chromatograph/Triple Quadrupole Mass Spectrometer

Table 6: Original OF Cannabinoid Method Gradient

Time (min)	Water with 5mM Ammonium Formate and 0.1% Formic Acid (MPA)	Methanol with 0.1% Formic Acid (MPB)	Flow (mL/min)
0.0	50%	50%	0.5
1.0	50%	50%	0.5
5.0	35%	65%	0.5
8.0	5%	95%	0.5
10.0	5%	95%	0.5

Table 6 displays the parameters for the OF cannabinoid gradient before any modifications. Several modifications were attempted to improve resolution and eliminate co-elution. In part, a change in the analytical column stationary phase or change in mobile phase composition can affect linearity and resolve interferences.[38]

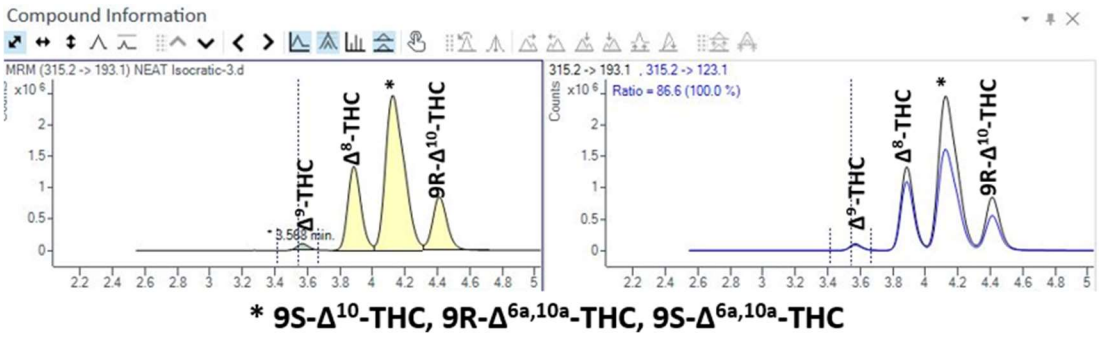

Figure 6: Complete Isocratic Method (20% MPA, 80% MPB) on Agilent 6460 Liquid Chromatograph/Triple Quadrupole Mass Spectrometer

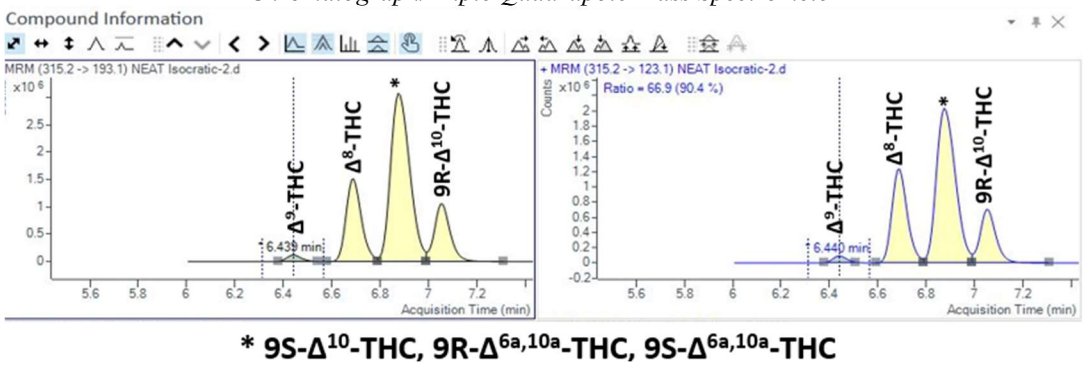

Figure 7: Isocratic Segment Between Six to Nine Minutes on Agilent 6460 Liquid Chromatograph/Triple Quadrupole Mass Spectrometer

The first aspect evaluated was a complete isocratic method using 20% MPA and 80% MPB resulted in baseline resolution but little separation between peaks. An isocratic segment between six to nine minutes was then attempted which provided the same results as a fully isocratic method. Chromatograms can be seen in Figures 6-7.

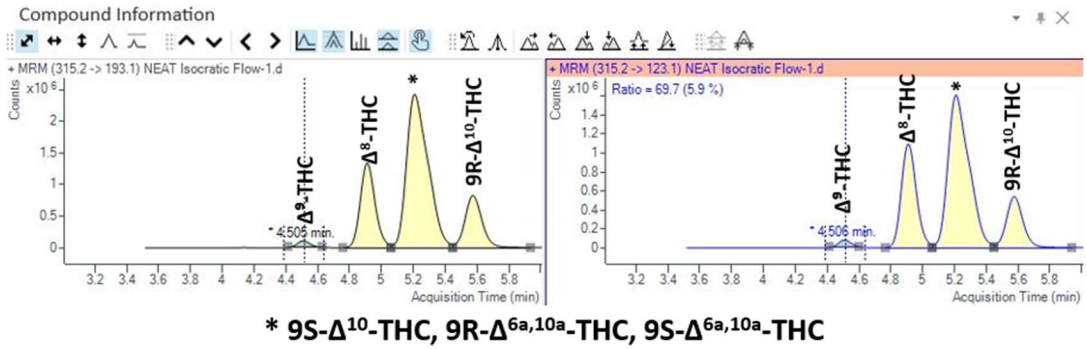

Figure 8: Flow Rate (0.4 mL per minute) Under an Isocratic Segment on Agilent 6460 Liquid Chromatograph/Triple Quadrupole Mass Spectrometer

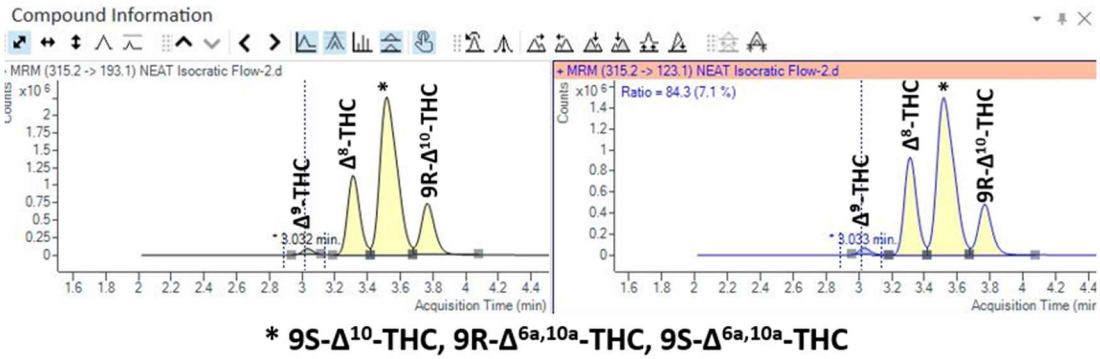

Figure 9: Flow Rate (0.6 mL per minute) Under an Isocratic Segment on Agilent 6460 Liquid Chromatograph/Triple Quadrupole Mass Spectrometer

Next, the isocratic flow rate was evaluated at 0.4 mL per min and 0.6 mL per min. The original method used a 0.5 mL per min flow rate. The chromatogram for the isocratic gradient segment at flow rates of 0.4 mL per min and 0.6 mL per min are shown in Figures 8 and 9. By increasing the flow rate to 0.6 mL per min, Δ^9-THC eluted at 3.014 minutes, whereas when the flow rate was set at 0.4 mL per min Δ^9-THC eluted a minute later at 4.478 minutes. Changing the flow rates provided little additional separation between Δ^9, Δ^8, and Δ^{10}-THC.

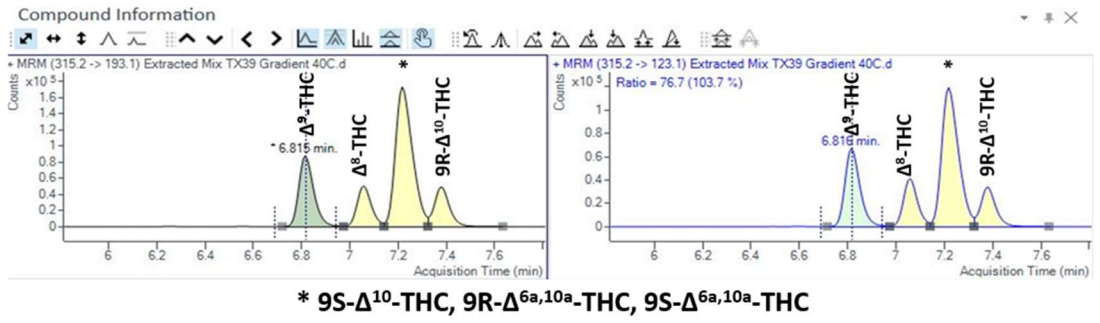

Figure 6: Blood Cannabinoids Gradient at 40°C on Agilent 6460 Liquid Chromatograph/Triple Quadrupole Mass Spectrometer

The column temperature was changed from 55° C to 40° C (Figure 10) keeping the blood cannabinoid gradient flow rate of 0.5 mL/min. Δ^9-THC eluted at 6.796 minutes

and demonstrated baseline resolution of Δ^8-THC. However, there was little improvement in the resolution.

Lastly, the gradient was changed from using 50% of MPA in the beginning of the analytical run to 70% of MPA, which is the gradient used for the ADFS Blood Cannabinoid LC/MS/MS SOP (Figure 11). Δ^9, Δ^8, and Δ^{10}-THC were retained until the composition of MPA was greater than that of MPB. This change involves a high percentage of MPB early in the analytical run which provided better resolution between Δ^9, Δ^8, and Δ^{10}-THC. Ending the run at 99% MPB ensures all targets were removed from the column. Baseline resolution between Δ^9, Δ^8, and 9R-Δ^{10}-THC was achieved. Co-elution still occurred between 9S-Δ^{10}-THC and both isomers of Δ^{6a10a}-THC. Table 7 shows post-modification results after the gradient change.

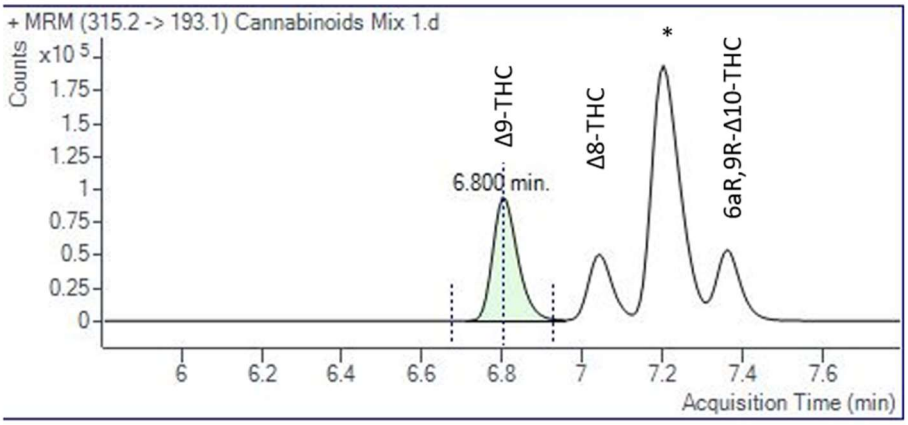

Figure 7: TIC of Δ^9, Δ^8, and Δ^{10}-THC Isomers after Gradient Modification

Table 7: Interference Results (Post-Modification)

Targets	Post-Modification Interference Occurred (Yes/No)	Post-modification If so, what target?
Δ^9-THC	No	
Δ^8-THC	No	
(6aR,9s)-Δ^{10}-THC	Yes	9S - $\Delta^{6a,10a}$ –THC 9R - $\Delta^{6a,10a}$ – THC
(6aR,9R)-Δ^{10}-THC	Yes	9S - $\Delta^{6a,10a}$ –THC (6aR,9s)-Δ^{10}-THC
9R - $\Delta^{6a,10a}$ – THC	No	
9S - $\Delta^{6a,10a}$ – THC	Yes	(6aR,9s)-Δ^{10}-THC 9R - $\Delta^{6a,10a}$ – THC
Synthetic Cannabinoids Tier 1 Mix	No	
Synthetic Cannabinoids Tier 2 Mix	No	

The final total ion chromatogram (TIC) (Figure 12) after gradient adjustment demonstrates the baseline resolution between Δ^9, Δ^8, 9R-Δ^{10}-THC, and other evaluated targets. Co-elution still occurred between 9S-Δ^{10}-THC and both isomers of Δ^{6a10a}-THC, Δ^9-THC-OH and Δ^8-THC-OH, and Δ^9-THC-O and Δ^8-THC-O. For reporting purposes, based on the data presented, 9S-Δ^{10}-THC and both isomers of Δ^{6a10a}-THC will be reported using an "or" statement, and Δ^9-THC-OH and Δ^8-THC-OH will be non-isomer specific, reported out as "THC-OH." Δ^8-THC-COOH and Δ^9-THC-COOH were fully resolved. Δ^{10}-THC-OH and Δ^{10}-THC-COOH were not available at the time of this study.

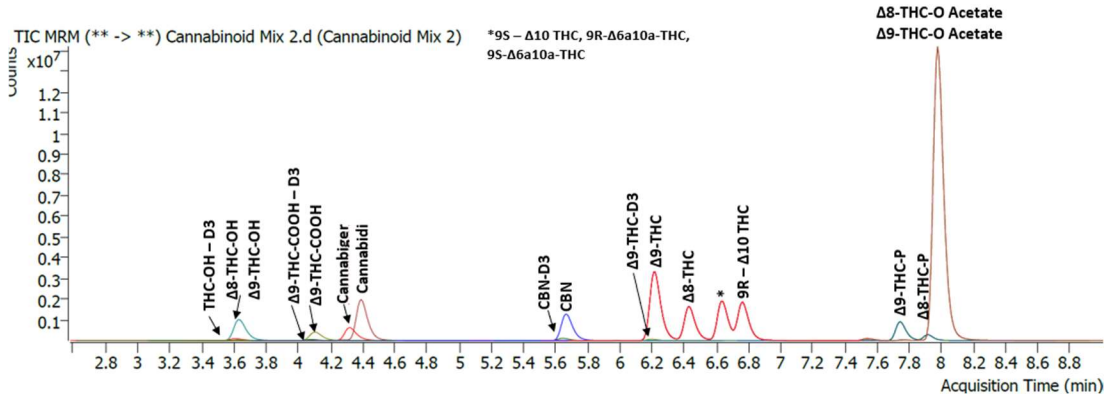

Figure 8: TIC of Validated Targets and Evaluated Targets Under Adjusted Gradient

Validation of Δ^8-THC and Δ^{10}-THC

A validation for the addition of Δ^8-THC to the existing OF cannabinoid method was conducted. Interference, carryover, and robustness were evaluated. For sensitivity, LOD was assessed using Immunalysis Synthetic Negative Saliva (Immunalysis Panoma, CA) fortified with Δ^8-THC and Δ^{10}-THC in duplicate over four days. Δ^8-THC was tested at 1.0 and 2.0 ng/mL and Δ^{10}-THC was tested at 1.0, 2.0, and 4.0 ng/mL. At least 75% of replicates passed at the determined LOD concentration of 1.0 ng/mL. Identification criteria for LOD studies recommended by ANSI/ASB Standard 036 for Method Validation in Forensic Toxicology are the following: a retention time ± 3% of the retention time of a standard, qualifier ratio: ± 20% compared to a standard, S/N: > 10, and adequate peak shape. Having a S/N > 10 was selected in this study as the required criteria instead of having a S/N > 3 as a conservative measure. All three targets evaluated at 1.0 and 2.0 ng/mL met the acceptance criteria. The results for the replicates of Δ^8 and Δ^{10}-THC over the four days batches were run are shown in Tables 8-10. If isomers met the acceptance criteria, a "Yes" was placed under the respective column. An LOD of 1.0 ng/mL had ≥ 75% of replicates pass all criteria for the concentrations evaluated. S/N in

three of the negative controls were > 3 for 9S-Δ^{10}-THC and Δ^{8}-THC, where poor chromatography was present. Therefore, these samples were deemed negative. The chromatograms for those samples with poor chromatography are provided in Appendix D. No carryover was observed after the 300 ng/mL calibrator for the previously validated targets, Δ^{8} and Δ^{10}-THC. However, blanks will continue to be injected between case specimens due to the regular occurrence of extremely high Δ^{9}-THC concentrations in casework. Robustness was evaluated by having two scientists participate in the extraction for four batches. Table 11 depicts final LOD concentrations for both Δ^{8} and Δ^{10}-THC and Figures 13 and 14 show the TIC of Δ^{8} and Δ^{10}-THC at 1.0 ng/mL.

Table 8: Δ^{8}-THC LOD Replicate Criteria Results

Date	Negative Control (S/N)	RT/Ratios Acceptable	2.0 ng/mL (S/N)	RT/Ratios Acceptable	1.0 ng/mL LOD (S/N)	RT/Ratios Acceptable
7/22/2021	3.0	Yes/Yes	256	Yes/Yes	163	Yes/Yes
7/22/2021			176	Yes/Yes	199	Yes/Yes
7/28/2021	2.0	Yes/No	276	Yes/Yes	253	Yes/Yes
7/28/2021			770	Yes/Yes	170	Yes/Yes
8/10/2021	1.0	Yes/No	63	Yes/Yes	20	Yes/No
8/10/2021			24	Yes/Yes	14	Yes/Yes
9/30/2021	1	Yes/Yes	286	Yes/Yes	102	Yes/Yes
9/30/2021			190	Yes/Yes	6	Yes/Yes

Table 9: 9S-Δ^{10}-THC LOD Replicate Criteria Results

Date	Negative Control (S/N)	RT/Ratios Acceptable	4.0 ng/mL (S/N)	RT/Ratios Acceptable	2.0 ng/mL (S/N)	RT/Ratios Acceptable	1.0 ng/mL (S/N)	RT/Ratios Acceptable
11/5/2021	1.0	Yes/No	515	Yes/No	397	Yes/No	215	Yes/Yes
11/5/2021			1213	Yes/Yes	251	Yes/Yes	151	Yes/Yes
11/19/2021	1.0	Yes/No	1416	Yes/Yes	238	Yes/Yes	418	Yes/Yes
11/19/2021			786	Yes/Yes	648	Yes/Yes	455	Yes/Yes
12/1/2021	6	Yes/Yes	1678	Yes/Yes	960	Yes/Yes	210	Yes/Yes
12/1/2021			∞	Yes/Yes	690	Yes/Yes	634	Yes/Yes
12/4/2021	3	Yes/Yes	583	Yes/No	392	Yes/Yes	278	Yes/Yes
12/4/2021			554	Yes/Yes	953	Yes/Yes	407	Yes/Yes

Table 10: 9R-Δ^{10}-THC LOD Replicate Criteria Results

Date	Negative Control (S/N)	RT/Ratios Acceptable	4.0 ng/mL (S/N)	RT/Ratios Acceptable	2.0 ng/mL (S/N)	RT/Ratios Acceptable	1.0 ng/mL (S/N)	RT/Ratios Acceptable
11/5/2021	0.0	Yes/No	591	Yes/Yes	452	Yes/Yes	221	Yes/Yes
11/5/2021			1233	Yes/Yes	249	Yes/Yes	145	Yes/Yes
11/19/2021	1.0	Yes/No	1341	Yes/Yes	226	Yes/Yes	407	Yes/Yes
11/19/2021			735	Yes/Yes	627	Yes/Yes	421	Yes/Yes
12/1/2021	2	Yes/Yes	1596	Yes/Yes	873	Yes/Yes	198	Yes/Yes
12/1/2021			1400	Yes/Yes	611	Yes/Yes	616	Yes/Yes
12/4/2021	1	Yes/Yes	619	Yes/Yes	382	Yes/Yes	262	Yes/Yes
12/4/2021			631	Yes/Yes	397	Yes/Yes	378	Yes/Yes

Table 11: Final LOD's for New Novel Cannabinoids

Target	Internal Standard	Limit of Detection (LOD)
Δ^8-THC	Δ^9-THC-D3	1.0 ng/mL
9R-Δ^{10}-THC	Δ^9-THC-D3	1.0 ng/mL
9S-Δ^{10}-THC	Δ^9-THC-D3	1.0 ng/mL

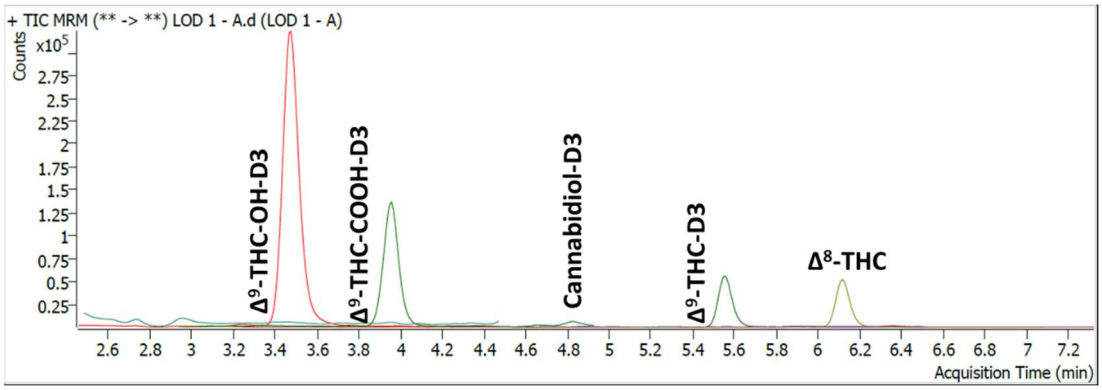

Figure 9: TIC of Final Δ^8-THC LOD of 1.0 ng/mL

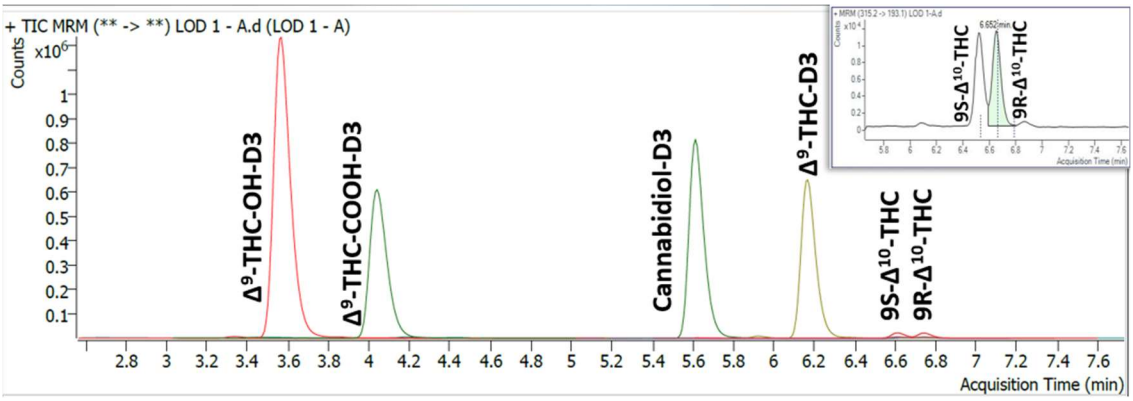

Figure 10: TIC of Final Δ[10]-THC LOD of 1.0 ng/mL

Interference was also evaluated by analyzing the batches containing calibrators and controls. No interferences were detected with previously validated targets. Δ^{10}-THC interference was described in previous section (Figure 12). The validation resulted in suitable identification of Δ^{8} and Δ^{10}-THC, qualitatively. Matrix interference for Δ^{8}-THC was also evaluated by analyzing 21 negative samples from different sources including expectorate, Quantisal (Immunalysis Panoma CA), and Oral-Eze devices (Thermo Fisher Auburn, AL) to determine if an endogenous interference was present. For Δ^{10}-THC, only seven Immunalysis synthetic OF samples were evaluated and no interferences were detected.

Stability of Δ^{8}, Δ^{9}, and Δ^{10}-THC at 20 °, 4 °, and -20 °C

The criteria for stability requires the tested concentration to be within ±20% of the T0 concentration of the sample. Six simulated case samples were spiked at an analytical concentration of 25 and 100 ng/mL each and were stored room temperature (20-22 °C), cooler (4 °C), and freezer (-20 °C) and evaluated at time-zero (T0), two weeks, one month, 60 days, and 90 days to determine how long validated targets as well as novel

cannabinoids were stable. Figures 15-20 show the individual targets' concentration difference between Δ^8, Δ^9, and Δ^{10}-THC from T0 to 90 days.

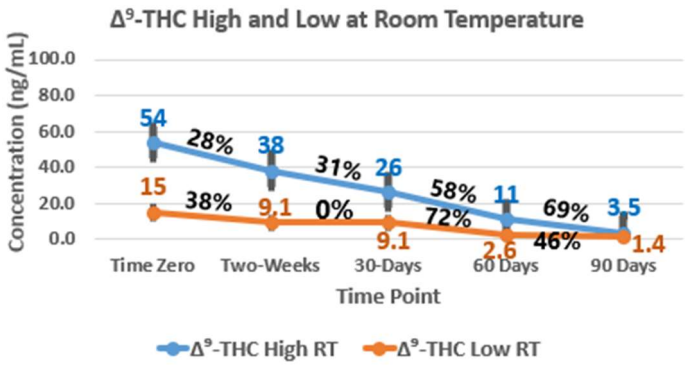

Figure 11: Δ^9-THC Stability Over 90 Days at Room Temperature

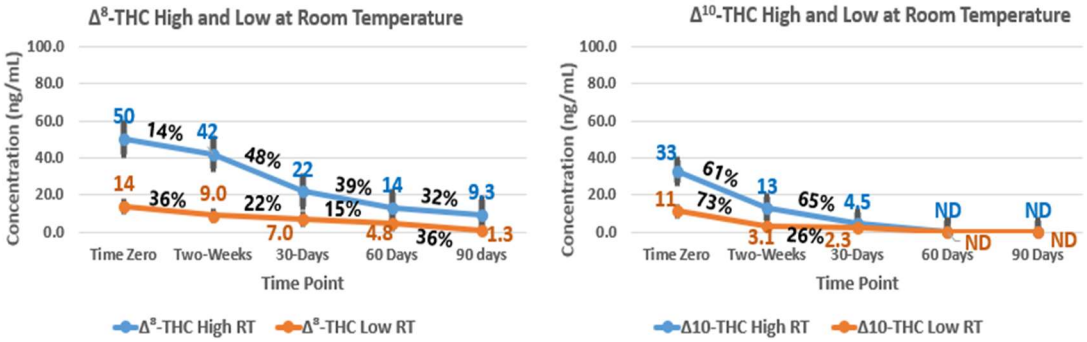

Figure 12: Δ^8 & Δ^{10}-THC Stability Over 90 Days at Room Temperature

The T0 concentrations were 15 and 50 ng/mL. This was about 50% less than the prepared concentrations, which is consistent with other studies.[39] Overall target stability at room temperature was poor, as Δ^8, Δ^9, and Δ^{10}-THC concentrations at room temperature at two weeks decreased by more than 20% for all samples, except for Δ^8-THC at high concentrations. Δ^{10}-THC could no longer be detected at 60 days. An overall decrease of 90-93% for Δ^9-THC and 81-90% for Δ^8-THC from T0 to 90 days was observed. Figures 15 and 16 show the percent change between each time point.

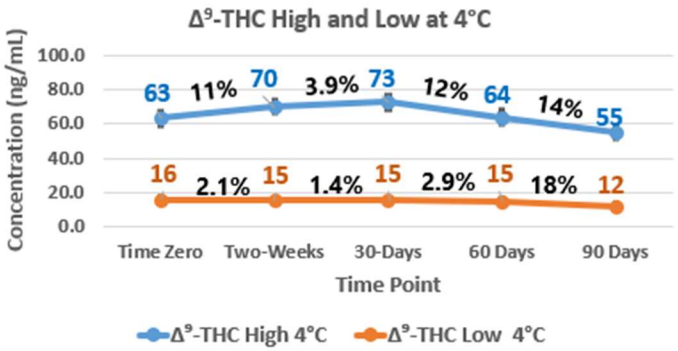

Figure 13: Δ[9]-THC Stability Over 90 Days at 4 ºC

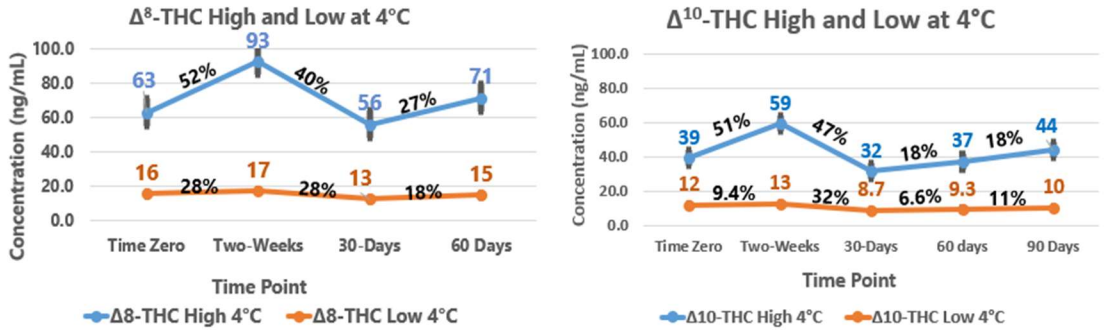

Figure 14: Δ[8] & Δ[10]-THC Stability Over 90 Days at 4 ºC*

*Δ[8]-THC samples had low recoverie so a 90 day concentration could not be determined accurately

Samples stored at 4 ºC were more stable than the room temperature samples, with Δ[9]-THC having exceptional stability for up to 90 days at both high and low concentrations. All targets showed an increase in concentration, but significantly larger increases were detected for Δ[8]-THC and Δ[10]-THC between T0 and two weeks. This will be explored in further studies. An overall decrease of 12-23% was observed for Δ[9]-THC concentrations and 11-12% for Δ[10]-concentrations from T0 to 90 days. The overall percent change for Δ[8]-THC could only be calculated up to 60 days which was 7.7-20%. All percent changes from T0 through 90 days did not exceed ±20%. Figures 17 shows the overall enhanced stability with Δ[9]-THC. Figure 18 shows the increases mentioned above from T0 to two weeks, and 30 days to 60 days. It is important to note that due to low

recovery at 4 ºC, 90-day concentrations could not be accurately determined for Δ^8-THC samples and are therefore not plotted in Figures 18 and 20.

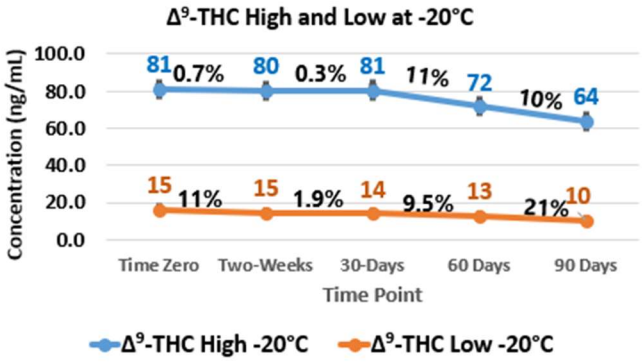

Figure 15: Δ^9-THC Stability Over 90 Days at -20 ºC

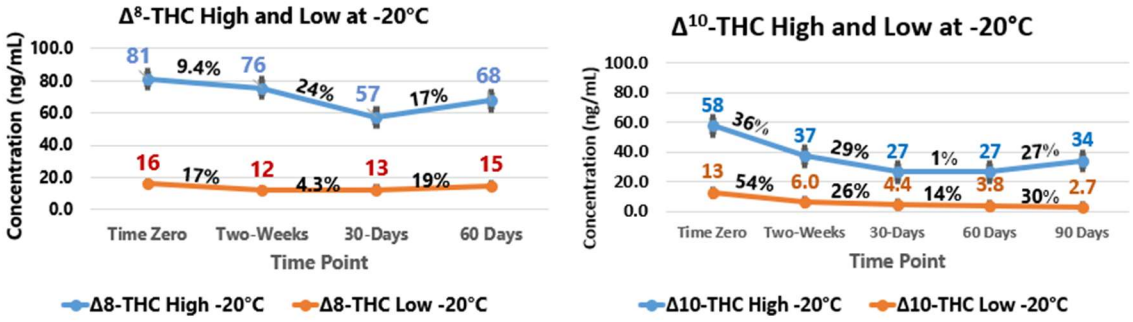

Figure 16: Δ^8 & Δ^{10}-THC Stability Over 90 Days at -20 ºC*

*Δ^8-THC samples had low recoveriesso a 90 day concentration could not be determined accurately

Lastly, all targets at -20 ºC had enhanced stability compared to room temperature, but were less stable than at 4 ºC. Δ^9-THC (Figure 19) still showed consistent stability and concentration in both high and low concentrations over 90 days. Similar to Δ^8 and Δ^{10}-THC (Figure 20) at 4 ºC, samples did decrease and begin increasing again at 30 days. However, Δ^{10}-THC at low concentrations had > 50% decrease from T0 to two weeks with steady decreases thereafter, no increases in concentration were observed. Δ^9-THC had an overall decrease between 21-37% with Δ^{10}-THC having a 41-79% decrese from T0 to 90

days. Low recovery also occurred in Δ^8-THC samples at -20 °C as well, so only up to 60 days overall change could be calculated, which was 4-20%.

Stability Casework Re-analysis

Table 12: Δ^9-THC DUI Concentration Difference Over 2.5 Years

Sample ID	Original (ng/mL)	2.5 Years (ng/mL)	% Change
19-4	62	36	-42%
19-5	150	116	-23%
19-6	42	34	-20%
19-14	7.0	6.2	-11%
19-16	110	95	-13%
19-17	41	35	-14%
19-20	290	268	-8%
19-21	400	294	-27%
19-22	55	45	-19%
19-24	52	46	-11%
19-27	14	10	-28%
19-29	9.8	8.7	-12%
19-30	82	69	-15%
19-31	14	0.5	-96%
19-32	61	45	-27%
18-3	69	14	-79%
18-4	102	32	-68%
	Median Decrease	**20% (Range: 8%-96%)**	

Previously analyzed cases were analyzed at two and half years to determine if the stability of Δ^9-THC was within 20% of the original concentration. Table 12, above, shows the concentration difference from the first analysis to the time point analyzed.

An evaluation of the number of days between a sample shipment and receipt at the laboratory was performed. Table 13 displays the important time points for a sample submitted for analysis. There is a median time of two days from shipment to receipt by the laboratory. However, there is a maximum of 95 days between collection and the first analysis. The stability studies showed that Δ^9-THC degrades quickly when stored at room temperature. Next, the data regarding the shipping carriers (e.g., USPS, UPS, FedEx) were further analyzed to determine if certain carriers took longer to deliver the samples (Table 14). All samples were packaged in the ADFS DUI biological specimen kits. Most samples were shipped through USPS Mail with a median of two days and an average of five days. There was an increase shipment time during the pandemic as staffing in postal services may have been reduced, which could have resulted in a higher-than-average shipping time. Table 15 displays the percentage of cases between <7 and > 90 days. 49% of cases received their first analysis between 30-60 days of collection. 73% of cases were collected and shipped in less than seven days with 82% of cases being received at the laboratory within seven days as well. 47% of cases were analyzed within the first month after being received at ADFS.

Table 13: Period of Time between Important Time Points for Evidentiary Sample

	Days Between Collection and Shipment	Days Between Shipment and Received	Days Between Collection and First Analysis	Days Between Received and First Analysis
N=	67	67	51	51
Median	3	2	38	25
Maximum	58	33	95	74
Minimum	1	0	5	0
Average	30	42	4	6
S.D.	6.4	4.9	19	16

Table 14: Days between Shipment and Received Dependent on Carrier

Days Between Shipment and Received			
	Median Days	**Average Days**	**Number of Samples**
US Mail	2	4.5	67
FedEx	1	1	2
UPS	4.5	4.5	8

Table 15: Percentage of Cases at Each Important Time Point for OF Specimens

% OF CASES	DAYS BETWEEN COLLECTION AND SHIPMENT (%)	DAYS BETWEEN SHIPMENT AND RECEIVED (%)	DAYS BETWEEN COLLECTION AND FIRST ANALYSIS (%)	DAYS BETWEEN RECEIVED AND FIRST ANALYSIS (%)
< 7 DAYS	73	82	2	4
7-15 DAYS	15	16	4	10
16-30 DAYS	9	0	27	47
31-60 DAYS	1.5	2	49	39
61-90 DAYS	1.5	0	16	0
>90 DAYS	0	0	2	0

DISCUSSION

Interference Comparison

With the emergence of novel cannabinoids, it is important for forensic laboratories to evaluate them under their current method conditions to determine if interference exists. If co-elution occurs, it can cause misidentification of targets and incorrect reporting. For instance, before modification of the OF cannabinoid gradient, Δ^9 and Δ^8-THC were resolved but did not have baseline separation. Similarly, the Δ^8 and Δ^{10}-THC isomers co-eluted. Without the ability to separate novel cannabinoids, scientists may incorrectly identify a detected compound as Δ^9-THC, which is controlled federally. This concern prompted the interference study and gradient adjustment described here.

Applying the blood cannabinoid gradient to the OF cannabinoid method, with initial conditions of 70% mobile phase B (MPB) (0.1% formic acid in methanol) and ending at 99% MPB, resulted in baseline resolution between the Δ^8, Δ^9, Δ^{10}-THC isomers. This separation was achieved by creating a shallower gradient in the congested region between six to nine minutes. Lin Lin (2020) published a validation for quantitating cannabinoids in OF, including Δ^8-THC. Unlike the ADFS method, the method used 0.1% formic acid in water/acetonitrile (95:5 v/v) (MPA) and 0.1% formic acid in acetonitrile (MPB) with a flow rate 0.5 L/min.[40] The amount of MPB also differed, compared to the

ADFS method, with the initial conditions of 55% MPB and ending conditions of 100% MPB, respectively. The method achieved baseline resolution between Δ^8 and Δ^9-THC.[40]

A report by Karaschner (2022) evaluated interference of Δ^8-THC and metabolites in blood and urine. There were differences in the instrument parameters such as ending conditions of 95% of 0.1% formic acid in acetonitrile (MPB) to ensure all phospholipids were eluted from the column compared to the ADFS OF method which had ending conditions of 99% MPB. Both studies, ADFS & Karaschner, used the same column, an Agilent 120 Infinity Lab Poroshell EC-C18.[41] Karaschner's method resulted in co-elution of Δ^8-THC-OH and Δ^9-THC-OH, but was able to achieve baseline resolution between Δ^8 and Δ^9-THC.

Due to co-elution between 9S-Δ^{10}-THC and both isomers $\Delta^{6a,10a}$-THC as well as co-elution between Δ^8 and Δ^9-THC-OH, reporting policies had to be evaluated with regards to results from the ADFS OF cannabinoid method. With Δ^8 and Δ^9-THC-OH co-eluting, the isomer specific nomenclature was dropped and will be reported out as "THC-OH." Due to co-elution of 9S-Δ^{10}-THC and both isomers of $\Delta^{6a,10a}$-THC, it will be reported as "9S-Δ^{10}-THC or $\Delta^{6a,10a}$-THC".

Comparison of Δ^8-THC Validations

As mentioned, there are few articles published regarding validation of Δ^8-THC and Δ^{10}-THC in OF. Chan-Hosokawa (2021) published an article reviewing the emergence of Δ^8-THC in DUI/D casework with blood as the target specimen. Unfortunately, an LOD could not be identified due to excessive blood matrix effects that were not compensated for by the internal standard (IS) chosen in the study, Δ^9-THC-D3.[42]

According to the author, if a matched deuterated internal standard was available, an LOD may have been identified. There was no matrix interference identified after evaluating 21 different OF matrices with the adjusted gradient in the ADFS OF cannabinoid method used in this study. This allowed the LOD for both Δ^8-THC and Δ^{10}-THC to be set at 1.0 ng/mL. Despite the fact that the ADFS method also used deuterated Δ^9-THC-D3 as the internal standard (instead of a deuterated version of Δ^8-THC and Δ^{10}-THC), an LOD was able to be identified most likely due to the lack of background interferences in oral fluid compared to blood.

Lin Lin also performed an accuracy and precision study to determine lower (LLOQ) and upper limits of quantitation (ULOQ). The LLOQ was set for their validation at 0.1 ng/mL and ULOQ at 800 ng/mL for both Δ^9-THC and Δ^8-THC.[43] ADFS is currently not quantitating Δ^8-THC, but the LOD for qualitative identification is set at 1.0 ng/mL. At ADFS, Δ^9-THC was previously determined to have an LLOQ of 1.0 ng/mL and a ULOQ of 300 ng/mL. In this study, an evaluation of an LOD below 1 ng/mL for Δ^8 and Δ^{10}-THC was not attempted due to the lack of biologically relevant concentrations observed in casework below 1 ng/mL.

Approximately 22% of OF DUI cases received at ADFS between 2018 to June 2021 had a Δ^9-THC concentration between 0-4.99 ng/mL in samples. Overall, Δ^9-THC OF cases had a median concentration of 26 ng/mL (Figure 21). With an LOD set at 1.0 ng/mL, forensic laboratories will capture biologically relevant concentrations regularly observed in casework. An LOD set too high could result in missing cannabinoids at lower concentrations.

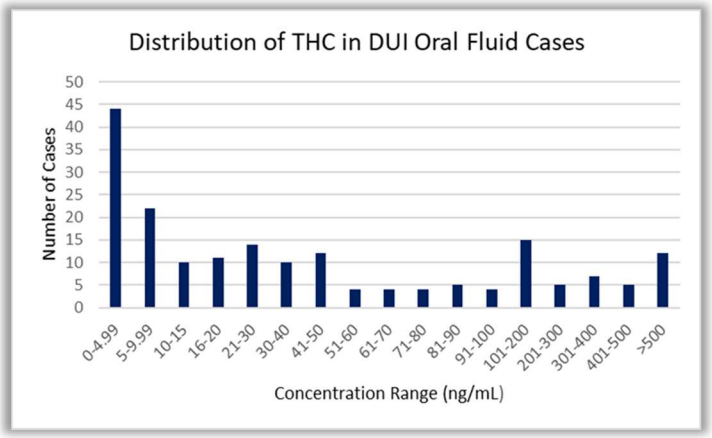

Figure 17: Δ^9-THC Distribution in DUI/D OF Cases Submitted to ADFS

Comparison of Δ^9-THC Stability Studies

Figure 19 contributed to the stability target concentration selections of 15 ng/mL, which was 50% the median concentration, and 50 ng/mL, which was two times above the median. This research was the first study to evaluate the stability of novel cannabinoids beyond 30 days in OF and provided many interesting observations. For example, Δ^8, Δ^9, and Δ^{10}-THC were not stable when stored at room temperature. In fact, Δ^{10}-THC could no longer be detected at 60 days. Overall, cannabinoid stability was greatly enhanced with refrigeration (4 ºC). Δ^9-THC showed excellent stability for up to 90 days, with overall target stability. Even at 90 days, the concentration was within 20% of the T0 concentration. Targets showed better stability at -20 ºC when compared to room temperature, but less stability than 4 ºC. Likewise, Δ^9-THC still was within 20% of the initial concentration after 90 days at -20 ºC. Another intriguing observation that warrants further studies was the slight increase in Δ^9-THC concentration at 4 ºC from T0 to two weeks.

These findings show that officers should be encouraged to ship OF samples in a timely fashion and/or store them under refrigeration until they are able to do so. Laboratories should store OF samples at 4 °C and it is important to note that storage stability for each specimen type (e.g. OF, blood, urine) may vary.

Table 16: Comparison of the Stability of Δ^9-THC Concentrations at Room Temperature Among This Study and Other Studies

Author	Specimen	Time Zero	7 Days	14 Days	1 Month	60 Days	90 Days
ADFS (High Conc.)	OF	0%	-	-28%	-52%	-80%	-94%
ADFS (Low Conc.)	OF	0%	-	-39%	-39%	-83%	-91%
Immunalysis[44] (High Conc.)	OF	0%	-30%	-30%	-60%	-	-
Immunalysis[44] (Low Conc.)	OF	0%	-25%	-27%	-30%	-	-
Moore[45] (Low Conc.)	OF	0%	-18%	-20%	-	-	-
Cohier[46] (High Conc.)	OF	0%	-14%	-30%	-	-	-

- Not Analyzed

Table 17: Comparison of the Stability of Δ^9-THC Concentrations at 4 °C Among This Study and Other Studies

Author	Specimen	Time Zero	7 Days	14 Days	1 Month	60 Days	90 Days
ADFS (High Conc.)	OF	0%	-	+11%	+16%	+1.6%	-13%
ADFS (Low Conc.)	OF	0%	-	-2.1%	-3.5%	-6.3%	-23%
Immunalysis[44] (High Conc.)	OF	0%	-	-20%	-10%	-	-
Immunalysis[44] (Low Conc.)	OF	0%	-	0%	-2%	-	-
Moore[45] (Low Conc.)	OF	0%	-14%	-10%	-	-	-
Cohier[46] (High Conc.)	OF	0	-2.2%	+1.4%	-	-	-
Meneses[48] (High Conc.)	Blood (Antemortem)	0%	-2%	-2%	+10%	+5%	+20%
Meneses[48] (Low Conc.)	Blood (Antemortem)	0%	+10%	10%	+10%	+10%	+19%

- Not Analyzed

Table 18: Comparison of the Stability of Δ9-THC Concentrations at -20 °C Among This Study and Other Studies

Author	Specimen	Time Zero	7 Days	14 Days	1 Month	60 Days	90 Days
ADFS (High Conc.)	OF	0%	-	-0.7%	-0.4%	-12%	-21%
ADFS (Low Conc.)	OF	0%	-	-11%	-13%	-21%	-37%
Cohier[46] (High Conc.)	OF	0%	-3.9%	-13%	-	-	-
Meneses[48] (High Conc.)	Blood (Antemortem)	0%	-40%	-80%	-95%	-98%	-98%
Meneses[48] (Low Conc.)	Blood (Antemortem)	0%	-5%	-35%	-35%	-50%	-55%

- Not Analyzed

The results of the stability study were compared to those from previous results (Tables 16-18). Immunalysis' storage and stability study was used as the model for the stability assessment. In their study, samples were stored at room temperature (in the dark) and 4 ºC and were analyzed in duplicate at time-zero, seven days, two weeks, and one month. Samples were spiked 50% below and 150% above the cutoff concentration of 4 ng/mL. The 2 ng/mL samples underwent a 30% loss in concentration between time-zero to one week and a decrease of 60% from two weeks to 30 days, respectively.[44] This agrees with our results as we had a 38% decrease from time-zero to two weeks. The samples spiked at 150% above cutoff (6 ng/mL) also had similar results to this study with a 30% decrease compared to 28%. The trend was a slightly larger percent loss in this study. A possible contribution to a larger percentage lost at low concentrations could be the specimens were not stored in darkness in this study, in contrast to the Immunalysis study.

Moore evaluated Δ^9-THC stability in OF samples spiked at 8 ng/mL, the approximate concentration for samples from marijuana users after smoking.[45] Δ^9-THC

was stable up to 14 days both at room temperature and under refrigeration. The Δ^9-THC concentration after extraction from the collection pad also resulted in concentrations lower than the initial concentrations of the spiked samples. Samples stored at 2-8 °C had only a 10% decrease from original concentration at two weeks. In comparison, room temperature conditions had losses of 20% at two weeks. The average decrease at room temperature in this study was 39% for low concentrations. The uncertainty of measurement for the analytical method is 18% for Δ^9-THC, so any increase or decrease in concentration with a magnitude less than 18% was not considered significant.

Cohier also examined storage conditions of room temperature, 4 °C, and -20 °C at seven and 14 days for the Quantisal and Certus collection devices. Samples extracted from the Quantisal were stable for up to 14 days at 4 °C and -20 °C. However, there was an overall decrease in Δ^9-THC concentration of ~ 30% at 14 days. Δ^9-THC concentrations in the Certus collector increased significantly at 14 days. It has been theorized that this increase could have been due to long lipophilic chain of the Δ^9-THC molecule binding strongly to the polyethylene collector pad.[46]

Crouch evaluated the Salivette, Intercept, Finger Collector, ORALscreen, and Hooded collection devices and found samples extracted from the Intercept device had less instability than when the Quantisal device was used. The Intercept samples stored at -20 °C, had a 21% decrease in Δ^9-THC concentrations over six weeks. A decrease of 20% is within the expected variability of the experiment, given that the devices may not collect exactly one milliliter of sample and through normal analytical variability.[47] At 4 °C, Δ^9-THC concentrations decreased by 45% in 2 weeks and deteriorated to 87% by six weeks. At room temperature, the concentration was 39% at 2 weeks and decreased to

86% of the initial concentration by 6 weeks. The chemical composition of the buffer in the collection could be a factor in the stability of the drug before first analysis occurs. Unlike the Intercept device, the sample concentration of samples collected with the Quantisal device had improved Δ^9-THC stability in this study as well as the others mentioned above.

Stability studies among different matrices may differ from those in OF, but share some similarities. Meneses evaluated Δ^9-THC stability in antemortem blood samples, fortified at concentrations at 20 and 50 ng/mL, from T0 up to 196 days. Samples had greater than 50% loss of the original concentration in samples with an initial 20 ng/mL concentration after 126 days.[48] By comparing the Meneses results with this study, it appears that Δ^9-THC is more stable in OF than blood at high concentrations (Table 18). Similar concentration increases occurred at 4 °C for high cannabinoid (50 ng/mL) concentrations between T0 and 30 days. A calculated 15% increase occurred between T0 and two weeks and a similar 11% increase in this study. Meneses stated that some reasons for the possible rise in concentration could be due to the pH of the sample, which was not tracked, and the possibility of *in vitro* conversion of cannabinoids since Δ^9-THC, Δ^9-THC-OH, Δ^9-THC-COOH, CBN and CBD were pooled into one sample instead of separating them. With the pooled samples, *in vitro* conversion could not be evaluated.

Coulter and Wagner evaluated *in vitro* conversion during sample preparation and extraction at a pH of 2.0, which resulted in 5% conversion rate of CBD to Δ^9-THC conversion.[49] By raising the pH to 5.0 *in vitro* conversion was reduced to only 1%. With the elimination of the acid component in sample preparation, the extraction resulted in zero conversion but resulted in reduced analyte recovery.

Previously Analyzed Casework

The study of previously analyzed OF casework at ADFS was the first study to evaluate Δ^9-THC in OF samples past 30 days in authentic casework. Overall, there was a 20% median decrease in Δ^9-THC with an 30% average decrease. Stability of Δ^9-THC over 2.5 years remained within ±20% for 53% of cases. The change in concentration ranged from 8% to 99% decrease from the original concentration.

All OF cases received from 2018 to present (n =1127) were evaluated to determine the time between collection and when the samples were received at the lab. On average, it required 15 days to be received at the lab, with a median of 8 days. 100 of the most recent OF cases were then evaluated to determine the days between collection and shipment, days between shipment and received, days between collection and first analysis, and days between received and first analysis (Table 10).

It takes an average 30 days for an OF cannabinoids case to be complete upon receipt at the laboratory. In comparison to March 2020, when the first analysis was completed by 21 days. The time between collection and first analysis was 95 days in 2022. Some reasons for the extended time to analysis could be due to the COVID-19 pandemic and an increase in the number of cases received at the lab. From 2020 to 2021, there was an average increase of 25% cases per month from 2020. The number is still rising for 2022 as there was a 15% increase in January 2022 from cases received in January of 2021.

Limitations

Table 19 summarizes the number of replicates in the five cannabinoid stability studies. Our study was modeled after the Immunalysis study. Immunalysis manufactures the Quantisal collection device. However, other studies analyzed their samples in triplicate. Despite this difference, the results still fell in range of the other studies. However, a power statistical analysis should be performed to determine if there a statistical difference between two to three replicates.

Table 19: Number of Replicates Evaluated Among Other Stability Studies

Name of Paper	*Author*	*# of Replicates*	*Time Points Evaluated (T=days)*	*Storage Condition (°C)*	*Specimen*
Evaluating Cannabinoid Interference and Drug Stability in Oral Fluid for DUID Testing	Jasmine Maxwell	2	T0, T14, T30, T60, T90	RT, 4, -20	OF
Storage and Transportation of Drugs in OF using the Quantisal™ Collection System	Immunalysis[32]	2	T0, T7, T14, T30	4, 25,	OF
Illicit Drugs in OF: Evaluation of Two Collection Devices	Camille Cohier[35]	3	T0, T7, T14	RT, 4, -20	OF
Cannabinoid Stability in Antemortem and Postmortem Blood	Vanessa Meneses and Dani Mata[49]	3	T0, T8, T14, T21, T29, T35, T49, T63, T92, T119, T150, T196	4, -4	Blood
Stability of Δ^9 - tetrahydrocannabinol (THC) in OF using the Quantisal™ collection device	Christine Moore[33]	3	T1, T3, T7, T10, T14	2-8, 22-28	OF

Another limitation could have been the tendency for Δ^9-THC to stick to glass or plastic. In this study, samples were in glass for two hours before the Quantisal collection occurred. After the sample was collected in the plastic Quantisal tube for three hours, the sample was transferred to another plastic container for storage. Recovery of the target from the pad could be less than 100% and there may be loss during the extraction.

Studies have shown that recovery from a collection device is a greater issue for low concentrations of Δ^9-THC compared to high concentrations, as losses could cause false negatives.[50]

Δ^9-THC degrades significantly more at room temperature in plasma or blood, when stored in plastic containers. Research by Wong et al. described Δ^9-THC as a degradable molecule that is subject to degradation when exposed to oxidation, elevated temperatures, and active surfaces such as glass and plastic. [51] The authors theorized that the large decreases observed were due to the inability to successfully extract the drug due to irretrievable binding to the degrading proteins in the blood and serum sample during storage.

Christophersen looked at spiked whole blood (10 pmol/mL) samples and evaluated Δ^9-THC in plastic versus glass. [52] There was less Δ^9-THC loss in glass, with an average of 0.9% decrease. Polystyrene plastic had an average loss of 7.8%. The author theorized that Δ^9-THC may diffuse into the plastic during storage and bind to the plastic. The rate of which Δ^9-THC compounds diffuse into the plastic may depend on the chemical composition of the containers. Quantisal tubes are comprised of polypropylene plastic.

Lastly, the number of previously analyzed samples for the evaluation of stability were limited. DUI cases are stored for up to 24 months. ADFS policy states that material relating to reported cases may be used for research or validation purposes if the following criteria is met: the case has been adjudicated or has a district attorney authorization (release letter), is not be related to a traffic homicide investigation, and there be a minimum of 5 mL of the sample retained.[53] With many ADFS OF cases originating from

2019, much of the criteria for analysis was not met until August 2021. Therefore, this limited the sample size to only 17 cases.

CONCLUSION

In all, this study adds to the understanding of the potential for interference from new hemp derived cannabinoids, the stability of Δ^9-THC under different storage conditions, and provides recommendations for time between collection and analysis of OF for Δ^9-THC. A method to resolve novel cannabinoids in OF was validated. In the interference study, baseline resolution between Δ^8, Δ^9, and Δ^{10}-THCwas achieved. Validation of Δ^8 and Δ^{10} isomers demonstrated a limit of detection of 1 ng/mL and no carryover after the highest calibrator (300 ng/mL). This is the first study to evaluate Δ^8 and Δ^{10}-THC in OF and expands the literature of stability studies past 30 days for cannabinoids in OF.

The results regarding the stability of novel cannabinoids provide data than can be used to encourage officers to ship samples in a timely manner or store in refrigeration until sample can be shipped. Refrigeration is especially important for storing of samples as results demonstrated the instability of cannabinoids stored at room temperature with enhanced stability at 4°C. Once samples arrive at laboratories, it is recommended that OF samples be stored at 4°C. If samples are stored at -20°C, freeze/thaw repetitions between analyses may negate any enhanced stability provided by the lower temperature. Overall stability in low concentration cannabinoids proved to have better stability across all

storage conditions in comparison to those at high concentrations. Based on the results from this study, Δ^9-THC is more stable than Δ^8 and Δ^{10}-THC for up to 90 days when stored at 4°C. Stability was demonstrated even in samples first analyzed 2.5 years ago as these samples met the criteria of having a median concentration within ±20% of the initial concentration providing even more support that 4°C is the recommended storage condition for OF samples. Part of ensuring samples are shipped in a timely manner is through regional seminar trainings stressing to law enforcement officer that the shipment of OF samples within 24 hours of collection, if possible, is important.

Further studies should explore the difference in glass versus plastic in OF sample containers to determine if the storage container could contribute to an increase or decrease in cannabinoid concentrations and recovery over time. Also, studies in regard to pH and *in vitro* conversion should be evaluated as it may be a possible cause for the increases observed with high and low concentrations of Δ^9-THC between T0 and 30 days. Another aspect to explore would be the effect of different buffer solutions as they can differ in their surfactants, preservatives, antimicrobial agents, and pH, all of which can contribute to drug stability. Laboratories should be encouraged to evaluate stability among their perspective time of analysis for their specific specimen type in casework. As novel cannabinoids become more readily available in products available to the public, it is important for laboratories to expand their scope of analysis to test for these cannabinoids with their existing methods and to confirm that no interference exists.

LIST OF REFERENCES

[1] Cone, E. J. (1993). Saliva testing for drugs of abuse. *Annals of the New York Academy of Sciences, 694*(1 Saliva as a D), 91-127.doi:10.1111/j.17496632.1993.tb18346.x

[2] Van der Linden, T., Legrand, S. A., Silverans, P., & Verstraete, A. G. (2012). DUI/D oral fluid and blood confirmation compared in Belgium. *Journal of analytical toxicology*, *36*(6), 418–421. https://doi.org/10.1093/jat/bks038

[3] Moore, C. (2017). Cannabinoids in oral fluid: Identification and interpretation of analytical results. *Handbook of Cannabis and Related Pathologies*, 1007–1017. https://doi.org/10.1016/b978-0-12-800756-3.00120-4

[4] Crouch, D. J., Crouch, D. J., & Baudys, J. (n.d.). Evaluation of saliva/oral fluid as an alternate drug testing specimen - final report

[5] Langel, K., Gjerde, H., Favretto, D., Lillsunde, P., Øiestad, E. L., Ferrara, S. D., Verstraete, A. G. (2014). Comparison of drug concentrations between whole blood and oral fluid. *Drug testing and analysis*, *6*(5), 461–471. https://doi-org.ezproxy3.lhl.uab.edu/10.1002/dta.1532

[6] Moore, C. (2011). Oral fluid for workplace drug testing: Laboratory implementation. *Drug Testing and Analysis, 4*(2), 89-93. doi:10.1002/dta.322

[7] Tai C. Kwong, Barbarajean Magnani & Christine Moore (2017) Urine and oral fluid drug testing in support of pain management, Critical Reviews in Clinical Laboratory Sciences, 54:6, 433-445, DOI: 10.1080/10408363.2017.1385053

[8] Kunkel F, Fey E, Borg D, et al. Assessment of the Use of Oral Fluid as a Matrix for Drug Monitoring in Patients Undergoing Treatment for Opioid Addiction. J Opioid Manag. 2015;11:435–442

[9] Verstraete, A. (2005, June 10). Oral fluid testing for driving under the influence of drugs: History, recent progress and remaining challenges. Retrieved March 21, 2021, from https://www.ncbi.nlm.nih.gov/pubmed/15944054

[10] Drummer, O. H. (2004). Postmortem Toxicology of Drugs of Abuse. Forensic Science International, 142(2-3), 101-113. doi:10.1016/j.forsciint.2004.02.013

[11] Oral fluid committee. (n.d.). Retrieved March 23, 2021, from https://www.soft tox.org/oral-fluid-committee

[12] Harper, C. E. (2020). *2020 soft/AAFS oral fluid committee survey - Soft-Tox.org*. Society of Forensic Toxicology. Retrieved April 1, 2022, from http://soft-tox.org/files/OF_DUID_2020.pdf

[13] Yartsev, A. (2017, October 6). Factors which determine the lipid solubility of drugs. Deranged Physiology. Retrieved April 20, 2021, from https://derangedphysiology.com/main/cicm-primary-exam/required-reading/pharmacokinetics/Chapter%201.3.0.3/factors-which-determine-lipid-solubility-drugs

[14] Society of Forensic Toxicology. 2018. ORAL FLUID SUB-COMMITTEE DUI/D SOFT-AAFS FREQUENTLY ASKED QUESTIONS (FAQ). February 24th. http://soft-tox.org/files/2018%20OF_FAQ_FINAL.pdf

[15] Crouch, D. J. (2005). Oral Fluid Collection: The neglected variable in oral fluid testing. *Forensic Science International*, *150*(2-3), 165–173. https://doi.org/10.1016/j.forsciint.2005.02.028

[16] AAA. (2020). Using Oral Fluid to Detect Drugs. Public Affair Resources AAA. Retrieved 2021, from https://publicaffairsresources.aaa.biz/wp content/uploads/2020/07/AAA-Oral-Fluid-Drug-Screening-Handout.pdf

[17] Loesche WJ, Bromberg J, Terpenning MS et al (1995). Xerostomia, xerogenic medications and food avoidances in selected geriatric groups. J Am Geriatr Soc 43: 401–407

[18] The Alabama Department of Forensic Sciences (2021, March 20). TOXICOLOGY ORAL FLUID DRUG TESTING PROGRAM. Retrieved from https://www.adfs.alabama.gov/services/tox/toxicology-oral-testing-program

[19] Society of Forensic Toxicology. 2020. Oral Fluid Drug Testing Pilot Project Guidelines for DUI/D Investigations. February 24th. http://softtox.org/files/2020_OF_Pilot121820.pdf

[20] Quantisal™. (2020, May 01). Retrieved March 21, 2021, from https://immunalysis.com/products/oral-fluid/quantisal

[21] Toxicology Specimen Collection Instructions: Antemortem

[22] Lin, L., Amaratunga, P., Reed, J., Huang, P., Lemberg, B. L., & Lemberg, D. (2020). Quantitation of Δ8-THC, Δ9-THC, cannabidiol and 10 other cannabinoids and metabolites in oral fluid by HPLC–MS-MS. Journal of Analytical Toxicology. https://doi.org/10.1093/jat/bkaa184

[23] Text - H.R.2 - 115th congress (2017-2018): Agriculture ... (n.d.). Retrieved January 25, 2022, from https://www.congress.gov/bill/115th-congress/house-bill/2/text

[24] Lin, L., Amaratunga, P., Reed, J., Huang, P., Lemberg, B. L., & Lemberg, D. (2020). Quantitation of Δ8-THC, Δ9-THC, cannabidiol and 10 other cannabinoids and metabolites in oral fluid by HPLC–MS-MS. Journal of Analytical Toxicology. https://doi.org/10.1093/jat/bkaa184

[25] Weed.com Admin. (2021, August 23). What is Δ 10 THC? Weed.com. Retrieved January 25, 2022, from https://weed.com/articles/cannabis-101/Δ-10-thc 2/what-is-Δ thc/#:~:text=How%20is%20Δ%2010%20THC%20Being%20 Made%3F%20Technically%2C,conditions%20that%20change%20the%20structu re%20of%20its%20compounds.

[26] Mallen, B. (2021, August 9). CBD & Delta 8 vs. THCO, THCP, HHC & Delta 10: Ultimate Cannabinoid Showdown. Secret Nature. Retrieved March 16, 2022, from https://secretnaturecbd.com/blogs/cbd/cbd-delta-8-vs-thco-thcp-hhc-delta-10-ultimate-cannabinoid-showdown

[27] THC-P effects: Everything you need to know. Herb. (n.d.). Retrieved March 16, 2022, from https://herb.co/learn/thc-p-effects-everything-you-need-to-know/

[28] Karmen Hanson, A. G. (2022, February 3). *State Medical Cannabis Laws*. Natural Conference of State Legislators. Retrieved March 5, 2022, from https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx

[29] American Nonsmokers' Rights Foundation. (2022, February 5). *State and local laws prohibiting smoking and vaping marijuana*. No Smoke. Retrieved April 1, 2022, from http://no-smoke.org/wp-content/uploads/pdf/marijuana-smokefree-laws-map.pdf

[30] ANSI/ASB Standard 036. (2019) Standard Practices for Method Validation in Forensic Toxicology - First Edition 2019. 1st edition. Approved American National Standard/American Academy of Forensic Sciences Standard Board, Colorado Springs, CO. http://www.asbstandardsboard.org/wp-content/uploads/2019/11/036_Std_e1.pdf (accessed Mar 05, 2022)

[31] Bickler, B. (n.d.). What is a chromatography gradient? Biotage. Retrieved January 25, 2022, from https://selekt.biotage.com/blog/what-is-a-chromatography-gradient

[32] Ventura, M., Pichini, S., Ventura, R., Zuccaro, P., Pacifici, R., de la Torre, R. (2007) Stability studies of principal illicit drugs in oral fluid: Preparation of reference materials for external quality assessment schemes. Therapeutic Drug Monitoring, 29, 662–665

[33] Immunalysis. (2017, May). Quantisal Oral Fluid Collection Device Storage and Transportation of Drugs in Oral Fluid [PDF]. Immunalysis Corporation

[34] Moore, C., Vincent, M., Rana, S., Coulter, C., Agrawal, A., & Soares, J. (2006). Stability of Δ9-tetrahydrocannabinol (THC) in oral fluid using the QUANTISAL™ Collection device. Forensic Science International, 164(2-3), 126–130. https://doi.org/10.1016/j.forsciint.2005.12.011

[35] Crouch, D. J. (2005). Oral Fluid Collection: The neglected variable in oral fluid testing. *Forensic Science International*, *150*(2-3), 165–173. https://doi.org/10.1016/j.forsciint.2005.02.028

[36] Cohier, C., Mégarbane, B., & Roussel, O. (2016). Illicit drugs in oral fluid: Evaluation of two collection devices. Journal of Analytical Toxicology, 41(1), 71–76. https://doi.org/10.1093/jat/bkw100

[37] The Alabama Department of Forensic Sciences. 2018. Toxicology Standard Operating Procedures 35 – Cannabinoids in Oral Fluid with LC/MS/MS. Hoover, Alabama, July, 18.

[38] ANSI/ASB Standard 036. (2019) Standard Practices for Method Validation in Forensic Toxicology - First Edition 2019. 1st edition. Approved American National Standard/American Academy of Forensic Sciences Standard Board, Colorado Springs, CO. http://www.asbstandardsboard.org/wp-content/uploads/2019/11/036_Std_e1.pdf (accessed Mar 05, 2022)

[39] Moore, C., Vincent, M., Rana, S., Coulter, C., Agrawal, A., Soares, J. (2006) Stability of Δ(9)-tetrahydrocannabinol (THC) in oral fluid using the Quantisal collection device. Forensic Sciences International,

[40] Lin, L., Amaratunga, P., Reed, J., Huang, P., Lemberg, B. L., & Lemberg, D. (2020). Quantitation of Δ8-THC, Δ9-THC, cannabidiol and 10 other cannabinoids and metabolites in oral fluid by HPLC–MS-MS. Journal of Analytical Toxicology, 46(1), 76–88. https://doi.org/10.1093/jat/bkaa184

[41] Karschner, E. (n.d.). The Rise of Δ8-THC in the United States. *TIAFT Bulletin*, *51*(2).

[42] A.Chan-Hosokawa, L. Nguyen, N. Lattanzio, W.R. Adams, Emergence of Δ-8 Tetrahydrocannabinol (THC) in DUI/D Investigation Casework: Method Development, Validation and Application, J. Anal. Toxicol. (2021)

[43] Lin, L., Amaratunga, P., Reed, J., Huang, P., Lemberg, B. L., & Lemberg, D. (2020). Quantitation of Δ8-THC, Δ9-THC, cannabidiol and 10 other cannabinoids and metabolites in oral fluid by HPLC–MS-MS. Journal of Analytical Toxicology, 46(1), 76–88. https://doi.org/10.1093/jat/bkaa184

[44] Immunalysis. (2012). *Storage and transportation of drugs in oral fluid*. SelectScience. Retrieved March 5, 2022, from https://www.selectscience.net/application-articles/storage-and-transportation-of-drugs-in-oral-fluid/?artID=56346

[45] Moore, C., Vincent, M., Rana, S., Coulter, C., Agrawal, A., Soares, J. (2006) Stability of Δ(9)-tetrahydrocannabinol (THC) in oral fluid using the Quantisal collection device. Forensic Sciences International, 164, 126–130

[46] Cohier, C., Mégarbane, B., & Roussel, O. (2016). Illicit drugs in oral fluid: Evaluation of two collection devices. *Journal of Analytical Toxicology*, *41*(1), 71–76. https://doi.org/10.1093/jat/bkw100

[47] Crouch, D.J. (2005) Oral fluid collection: the neglected variable in oral fluid testing. Forensic Sciences International, 150, 165–173

[48] Meneses, V., & Mata, D. (2019). Cannabinoid stability in ANTEMORTEM and postmortem blood. *Journal of Analytical Toxicology*, *44*(2), 126–132. https://doi.org/10.1093/jat/bkz073

[49] Coulter, C., & Wagner, J. R. (2021). Cannabinoids in oral fluid: Limiting potential sources of cannabidiol conversion to Δ9- and Δ8-tetrahydrocannabinol. *Journal of Analytical Toxicology*, *45*(8), 807–812. https://doi.org/10.1093/jat/bkab074

[50] Moore, C., Vincent, M., Rana, S., Coulter, C., Agrawal, A., Soares, J. (2006) Stability of Δ(9)-tetrahydrocannabinol (THC) in oral fluid using the Quantisal collection device. Forensic Sciences International, 164, 126–130

[51] Wong, A. S., Orbanosky, M. W., Reeve, V. C., & Beede, J. D. (1982). Stability of Δ-9- tetrahydrocannabinol in stored blood and serum. PsycEXTRA Dataset. https://doi.org/10.1037/e497082006-012

[52] Christophersen, A. S. (1986). Tetrahydrocannabinol stability in whole blood: Plastic versus glass containers. Journal of Analytical Toxicology, 10(4), 129–131. https://doi.org/10.1093/jat/10.4.129

[53] The Alabama Department of Forensic Sciences. 2021. Toxicology Standard Operating Procedure 7.4 – Handing of Test and Calibration Items. Hoover, Alabama, August, 202

APPENDIX A

OF Survey Questions

1. What is the name of your agency?
2. Does your laboratory perform DUID testing?
3. Which (evidentiary) specimen do you typically test in DUI/D cases?
4. Does you statute allow for Oral Fluid evidentiary (confirmation) drug testing in DUID cases?
5. If so, what terminology is used in your state statute?
6. Has your state/jurisdiction proposed a bill change to allow for evidentiary (confirmation) oral fluid drug testing?
7. Does your laboratory offer in-house oral fluid drug evidentiary (confirmation) testing in DUID cases?
8. If you perform oral fluid evidentiary (confirmation) testing, do you report results as quantitative or qualitative?
9. How does your laboratory assess carryover in your confirmation testing?
10. What collection device is used to collect oral fluid confirmation specimens?
11. If not fully implemented, has your laboratory started developing and/or validating oral fluid drug evidentiary (confirmation) testing?
12. If not, does your lab outsource oral fluid drug evidentiary (confirmation) testing to a reference laboratory (e.g. NMS Labs, Forensic Fluids) on a routine basis? [referring to casework, not pilot project samples]
13. Does your state statute allow for oral fluid roadside screening by law enforcement?
14. Has your state/jurisdiction conducted and completed an oral fluid pilot project?
15. If so, was it in conjunction with your DRE program?
16. If so, was it in collaboration with your Traffic Safety Resource Prosecutor?
17. If so, did it involve comparing oral fluid roadside devices to confirmation specimens?

18. Does law enforcement in your jurisdiction use oral fluid roadside screening devices to establish probable cause (e.g. similar to PBT for alcohol)?
19. Approximately how many OF screening devices are being used by law enforcement?
20. If so, which oral fluid roadside screening device(s)? [Select all that apply] (Draeger DT5000, Alere SoToxa (fka DDS2), Drug Wipe, Randox Multistat, N/A, Other)
21. Does your state have an approved list of roadside oral fluid screening devices (e.g. DT5000, SoToxa)?
22. Has your state/jurisdiction had a Daubert or Frye hearing related to roadside oral fluid testing (e.g. DT5000, SoToxa for probable cause)?
23. Has your state/jurisdiction had a Daubert or Frye hearing related to oral fluid testing evidentiary (confirmation) testing?
24. Are you familiar with the SOFT OF Committee?
25. Please provide more details regarding oral fluid drug testing in your state/laboratory.

Appendix B

List of Synthetic Cannabinoids

Table A1: List of Tier 1 and Tier 2 Synthetic Cannabinoids

Synthetic Cannabinoids 1	Synthetic Cannabinoids 2
4-Cyano-CUMYL-BUTINACA	5F-AEB
4F-MDMB-BUTINACA	5F-AMB
5F-ADB	5F-MDMB-PICA
5F-ADB-PINACA	5F-NPB-22
AB-FUBINACA	5F-PB-22
ADB-FUBINACA	AB-CHMINACA
AMB-CHMINACA	ADB-FUBICA
APP-BUTINACA	EMB-FUBINACA
FUB-AMB	MAB-CHMINACA
JWH-018	MDMB-FUBICA
MDMB-4en-PINACA	MMB-CHMICA
MDMB-CHMICA	MMB-FUBICA
MDMB-CHMINACA	
MDMB-FUBINACA	
MO-CHMINACA	
5F-MDMB-PICA	
5F-PB-22	

Appendix C

Quantisal Collection Instructions

ADFS provides law enforcement throughout the State with biological specimen kits that contain the Quantisal collection device as well as two grey stopper tubes for collection of blood. Initiation of Quantisal oral fluid sample collection should occur in the following order of timing preference: at the roadside (after 10-minute observation period), prior to DRE evaluation (if applicable), after DRE evaluation (if applicable), at the same time as the blood draw. Below is the list of instructions given to officers for collecting an oral fluid sample for DUI cases.

1. Check expiration date on Quantisal packaging and ensure the subject has refrained from smoking and consumption of food or beverage for 10 minutes prior to specimen collection.
2. Fill out Specimen Security Seal or label with subject's name, date & time of collection, and collector's initials.
3. Instruct the subject to move tongue side to side to accumulate oral fluid in his/her mouth to facilitate collection.
4. Put on gloves and wear throughout the collection process. Do not allow the subject to touch the collection device or tube.
5. Peel back and open package to remove collector (oral absorbent swab on plastic stick).
6. Place the position collector (oral absorbent swab) under the subject's tongue (like a thermometer). Instruct the subject to close his/her mouth, keep the tip of the device pointed down, and place head down, chin to chest to allow gravity to help with oral fluid collection.
7. Wait until the indicator turns BLUE or 10 minutes has elapsed. Note on submission form if indicator did not turn BLUE. Collection time may take from 2-10 minutes to collect approximately 1 mL of oral fluid.
8. Hold the red-capped tube with blue liquid in an upright position and uncap by pushing up with thumb(s). Retrieve collector (oral absorbent swab) from subject's mouth, place into the uncapped transport tube.
9. Snap cap firmly into tube for transport.
10. Mix saturated collector (oral absorbent pad) with the blue liquid by gently shaking the tube.
11. Seal top of collector with specimen security seal (or evidence tape). Initial and date seal.

Appendix D

Negative Control Chromatograms with a S/N >3

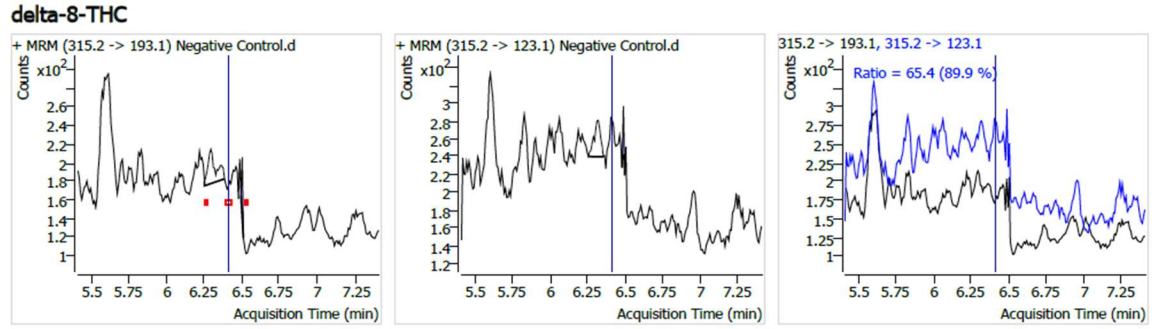

Figure C1: Poor Chromatography of Negative Control from 7/22/21

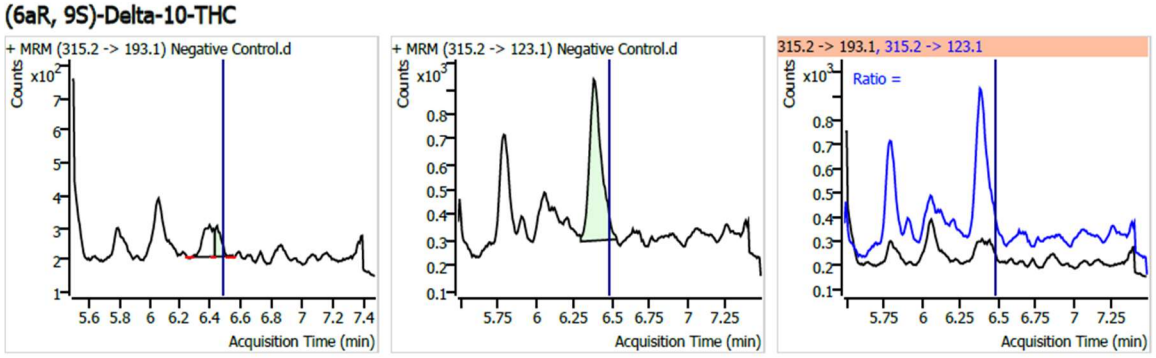

Figure C2: Poor Chromatography of Negative Control from 12/1/21

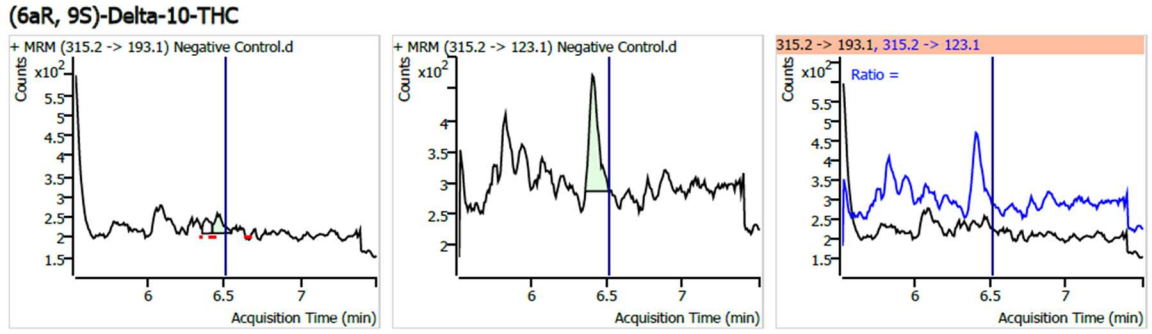

Figure C3: Poor Chromatography of Negative Control from 12/4/21