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Analyzing Cannabinoid Stability in Different Conditions and Validating Novel Cannabinoids for Oral Fluid Analysis

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ANALYZING CANNABINOID STABILITY IN DIFFERENT CONDITIONS AND VALIDATING NOVEL CANNABINOIDS FOR ORAL FLUID ANALYSIS

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FORENSIC SCIENCE

ABSTRACT

Oral fluid (OF) drug testing has been expanded to laboratory evidentiary confirmation testing in driving under the influence (DUI) cases. OF samples are collected by officers at the roadside using collection devices such as the Quantisal® device. Tetrahydrocannabinol (Δ^9-THC) was the most prevalent drug in Alabama DUI casework in 2022. Δ^9-THC remains a Schedule I drug in Alabama and federally. Current validated targets in cannabinoid oral fluid testing include Δ^9-THC, Δ^8-THC, 9R-Δ^{10}-THC, 9S-Δ^{10}-THC 11-hydroxy-Δ^9-THC (THC-OH), 11-nor-9-carboxy-Δ^9-THC (THC-OOH), cannabigerol (CBG), cannabidiol (CBD), and cannabinol (CBN).

Due to the 2018 Farm Bill, cannabinoids derived from hemp with a Δ^9-THC concentration less than 0.3% are legal in Alabama. This has caused novel cannabinoids such as tetrahydrocannabinol-acetate (THC-O) and tetrahydrocannabiphorol (THC-P) to be manufactured and sold legally. Methods need to be developed to ensure these legal cannabinoids can be distinguished from Δ^9-THC during confirmatory testing. This study validated THC-O and THC-P to add to the oral fluid extraction method in use at the Alabama Department of Forensic Sciences (ADFS).

Understanding cannabinoid stability is important as it is common for long periods of time to pass between collection of a sample and analysis. Several factors affect cannabinoid stability such as storage temperature, light conditions, and volume of solution a drug is in. This study examined how cannabinoid stability is affected when oral

fluid samples are stored at 20°C (room temperature), 4°C (refrigeration), -20°C, or in the trunk of a car at varying time points. Samples were also subjected to different light conditions during storage. Δ^9-THC concentrations were considered stable if the concentration at time of reanalysis was within 20% of the initial concentration. Average Δ^9-THC concentrations in OF remained stable after one month when samples were stored at 4°C or -20°C. Samples did not remain stable at 4°C when exposed to light or when samples were at low volumes prior to analysis. Storing samples in the trunk of a car led to Δ^9-THC instability after one week of storage at 31°C. Samples were stable in glass and plastic. Quantisal® buffer was stable when stored at 32°C for seven days prior to collection of OF.

Keywords: Oral Fluid, Stability, Cannabinoids, Validation, Quantisal®

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INTRODUCTION

Δ^9-THC Pharmacology and Implications

Marijuana is any part of the Cannabis Sativa plant that can produce psychoactive effects. Cannabinoids refer to the different compounds that are structurally similar to Δ^9-THC found within the flowers, leaves, stem, seeds, or roots of the Cannabis sativa plant. The main psychoactive compound from *Cannabis sativa* is Δ^9-Tetrahydrocannabinol (Δ^9-THC). When inhaled or ingested, Δ^9-THC acts on the cannabinoid receptors CB1 and CB2, producing psychological and physiological effects such as euphoria, hallucinations, lack of time perception, and impaired memory.[1] The effects of Δ^9-THC resemble the effects of central nervous system depressants and stimulants. Cannabis is a strictly controlled substance. Δ^9-THC remains a Schedule I drug federally and in Alabama. Suspects caught using cannabis may be imprisoned for one year and given up to a six thousand dollar fine upon their first conviction in Alabama.[2] Δ^9-THC use is widespread with users feeling they can get a high without resorting to drugs that come with more risks and potential for abuse.

Δ^9-THC blood concentrations peak during smoking and maximum effects from Δ^9-THC occur after peak blood concentration and as Δ^9-THC concentration begins to decrease. Δ^9-THC is rapidly eliminated from the blood before it saturates tissues which results in the pharmacological effects as the drug binds to the cannabinoid receptors in the body.[3] Δ^9-THC is lipid soluble which allows for fatty-tissues to absorb the drug

carried to different tissues by the circulatory system, including the brain, where Δ^9-THC acts on the receptors found on neurons.[4] Typically, Δ^9-THC is detected in blood and oral fluid, while the inactive metabolite, 11-nor-9-carboxy-THC (THC-COOH), is detected in urine after oxidation of the active metabolite 11-hydroxy-THC (THC-OH).[5] Natural cannabinoids that are routinely found in Δ^9-THC positive cases include cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN).

Δ^9-THC and its metabolites were the most prevalent drugs detected in 41% of DUI cases at the Alabama Department of Forensic Sciences in 2022.[6] In 2021, the use of marijuana for medical purposes was legalized in Alabama.[7] With the availability of medical marijuana in Alabama, that number will most likely rise even more in the coming years. It is important to understand the effects Δ^9-THC can have on the body and whether a person was impaired while operating a vehicle. Methods should be in place at crime laboratories across the country to detect Δ^9-THC, its metabolites, and novel cannabinoids in biological specimens to explain possible impairment during a DUI trial. Novel cannabinoids are designed as legal alternatives for users that desire a legal high.

Novel Cannabinoids

Novel cannabinoids (e.g., Δ^8-THC) are constantly evolving with the outcome of flooding the market with cannabinoids that have effects similar to Δ^9-THC.[8] The 2018 Farm Bill states that hemp and hemp-derived cannabinoids shall not be included in the definition of marijuana as stated in the Controlled Substances Act.[9] It also states that products derived from cannabis that have less than 0.3% Δ^9-THC are to be removed from the Controlled Substances Act. As a result, novel cannabinoids have started to appear in

illicit drug markets, including tetrahydrocannabiphorol (THC-P) and tetrahydrocannabinol-acetate (THC-O).[10] These two cannabinoids produce similar effects to that of Δ^9-THC, while remaining technically legal in Alabama because they are all derived from hemp. The structures of Δ^9-THC, Δ^9-THC-P, and Δ^9-THC-O can be seen in Appendix A. The Drug Enforcement Administration (DEA) recently released a statement stating that THC-O has been classified as a Schedule I substance since it is not naturally occurring and can only be produced synthetically.[11] Legislation is expected to be introduced in Alabama to consider making THC-O and other novel cannabinoids controlled substances.

With the passing of the 2018 Farm Bill, the production of novel hemp-derived cannabinoids has greatly increased. It is important for forensic laboratories to continuously adapt their cannabinoid methods to detect and identify these new targets. Cannabinoids that are not controlled, such as Δ^8-THC and Δ^{10}-THC, need to be correctly identified to ensure they are not mistakenly identified as and do not interfere with the analysis of Δ^9-THC or its metabolites. The legal status of novel cannabinoids is also subject to change. The legality of products containing THC-O and THC-P in Alabama is not clear at the time of this research as the DEA recently announced in a letter that it considers THC-O to be a Schedule I substance due to its synthetic nature. As the legislature changes, it is vital to be able to positively identify the targets THC-O and THC-P to make sure they are not misidentified as the currently illegal Δ^9-THC or its metabolites. Positive identification of THC-O is also necessary as it may be designated as a controlled substance in Alabama in the near future.

Δ^{10}-THC can be synthesized from Δ^{8}-THC, which is produced by the isomerization of CBD from the hemp plant.[12] Since hemp-derived CBD is the starting material, Δ^{10}-THC, when derived from hemp, would be excluded from the list of controlled substances in Alabama. Both stereoisomers of Δ^{10}-THC, 9R- Δ^{10}-THC and 9S-Δ^{10}-THC, are produced when synthesizing Δ^{10}-THC. The cannabinoid THC-O can be synthetically derived from CBD and it produces pharmacological effects about three times greater than Δ^{9}-THC.[13] Acetic anhydride is a highly flammable solvent used in the synthesis, which makes the synthesis of THC-O dangerous. CBD can be used as the starting material to make the naturally occurring THC-P, again making it exempt from being classified as a Schedule I substance. THC-P has a seven alkyl side chain that influences the pharmacological effects in the body.[14] Citti et al. found that THC-P is about thirty times more active than Δ^{9}-THC due to an increased binding affinity to the CB1 receptor. THC-P and THC-O can be converted into Δ^{8}-THC-O and Δ^{8}-THC-P, respectively or can be processed further to form Δ^{9}-THC-O and Δ^{9}-THC-P.

Drugs in Oral Fluid

Typical routes of administration for cannabis are through smoking or oral ingestion. Novel cannabinoids and Δ^{9}-THC are also being added to vape liquids. Ciolino et al. looked at 300 different vaping liquid products labeled to contain Δ^{9}-THC and found that 60% of those products contained low concentrations of at least one novel cannabinoid.[15] Because Δ^{9}-THC and other cannabinoids are smoked or taken orally, these drugs are present in the user's oral fluid.

Oral fluid consists of saliva produced by the salivary glands and other substances present in the mouth such as mucosal cells, food residue, and drug residue.[16] Once ingested, drugs collect in the oral fluid through passive diffusion into the epithelial cell membranes. Basic drugs such as cocaine are present in higher concentrations than in the blood due to ion trapping.[17] Ion trapping occurs when molecules diffuse across a membrane before becoming ionized due to the lower pH. The ionized molecule cannot diffuse back across the molecular membrane.[18] This also results in basic drugs (e.g. Δ^9-THC) to be detected for a longer time than in blood. The psychoactive compounds in cannabis can be detected in the blood and oral fluid of users.[19] Δ^9-THC concentrations in oral fluid are similar to that of blood concentrations, but may be higher right after smoking due to absorption of the drug in the oral cavity.[20] Consequently, Δ^9-THC concentrations in oral fluid may better represent the effects caused by Δ^9-THC on the user than in blood samples where counterclockwise hysteresis occurs. Blood and oral fluid both offer similar detection times for drug analysis. Positivity in both matrices indicate recent drug use.

Oral fluid and blood samples are typically preferred over urine samples for DUI testing in toxicology laboratories because urine shows the history of drug use. Drug detection times in urine are much longer than both blood and oral fluid. Drugs can be detected in the urine for up to several weeks and does not necessarily mean the user was under the influence of those drugs during the time of collection.[21] Drugs can be detected in blood and oral fluid for up to 24 hours and provide information about current drug use. Limits of detection can be adjusted to shorten this window.

Law enforcement in Alabama have been using roadside oral fluid screening devices when a subject is suspected to be driving under the influence of drugs (DUID) since 2018. According to the oral fluid toolkit sponsored by the AAA, oral fluid is a practical biological specimen used by field devices due to it being less invasive, faster, and simpler than collecting blood.[22] Roadside screening devices such as the Draeger DT5000 have been developed to screen oral fluid samples taken at the roadside. An amendment was made to the implied consent law in Alabama in 2021 which states that by signing their drivers licenses', vehicle operators have consented to giving blood or oral fluid to be tested for drugs for evidentiary testing at ADFS.[23] Once a roadside screen test shows a positive result, officers have probable cause to collect an oral fluid sample to be sent to the ADFS for confirmatory testing.

Oral fluid is also used for confirmatory drug testing in the laboratory. The Quantisal® device is the main collection device currently used to collect oral fluid samples for submission to the Alabama Department of Forensic Sciences (ADFS) for confirmatory testing. The Quantisal® collection pad is held under the tongue until the indicator turns blue or ten minutes has elapsed.[24] The device is designed to collect one milliliter of oral fluid. The pad is deposited into the Quantisal® device which contains about 3 mL of Quantisal® buffer, so the final volume in the Quantisal® tube is approximately 4 mL. Therefore, analytical methods need to be designed to account for the dilution of the drug during oral fluid collection.

The collection of oral fluid is a much less invasive method than for blood or urine samples. Suspected drug users are also more open to giving an oral fluid sample than blood.[25] Oral fluid testing is relatively new and quantitative analysis of oral fluid can be

challenging. Oral fluid testing can produce a higher uncertainty of measurement than blood due to collection volume of oral fluid samples. Another limitation of oral fluid is that some drugs can cause a dramatic decrease in oral fluid secretion making it difficult to the volume of saliva required for the analysis. Drugs that have anticholinergic activity against the M3 muscarinic receptor are most common cause of decreased saliva production.[26] Δ^9-THC and other cannabinoids are commonly found in polydrug use cases, which could lead to instances where the user does not produce much saliva. Limited oral fluid volume collections should be recorded (e.g. indicator did not turn blue) as it can make reanalysis difficult due to the limited amount of sample submitted. Van der Linden et al.[27] developed a formula to correct drug concentrations using the average weight of the collection device prior to collection and the weight of the device after collecting the oral fluid sample.

Existing Cannabinoids in Oral Fluid Extraction and Quantitation Methods

While cannabinoid confirmation in oral fluid testing is relatively new, several methods have been developed to accurately quantitate cannabinoids following extraction from oral fluid. All current methods use chromatography to separate the cannabinoids based on their boiling points, molecular mass, or polarity. The concentration of the cannabinoids is measured by mass spectrometry. Early methods used gas chromatography, which uses a gaseous mobile phase and a solid or liquid stationary phase. Verstraete determined that analysis by gas chromatography-mass spectrometry (GC/MS) following liquid extraction was not sensitive enough to separate and accurately report Δ^9-THC and its metabolites.[28] Currently, the gold standard for Δ^9-THC analysis is

liquid chromatography-mass spectrometry (LC/MS/MS) because it is a sensitive and efficient method. Laloup demonstrated that LC/MS/MS for Δ^9-THC in oral fluid quantitative analysis was effective and accurate while not requiring any of the sample cleanup or derivatization that GC methods require.[29] This study also showed that liquid-liquid extraction performed prior to analysis was very effective and led to decreases in matrix interference.

The method currently in use at ADFS is the standard operating procedure (SOP) TX35 – Cannabinoids in Oral Fluid with LC/MS/MS. Oral fluid samples suspected of containing cannabinoids will undergo liquid-liquid extraction before being analyzed with liquid chromatography – tandem mass spectrometry. The LC/MS/MS in use at ADFS are the Agilent 6460 and 6470 liquid chromatography triple quadrupole tandem mass spectrometers. Current validated targets for this method include Δ^9-THC, THC-OH, THC-COOH, Cannabidiol (CBD), Cannabinol (CBN), Cannabigerol (CBG), and Δ^8-THC. All validated targets must first have met validation guidelines set by the American Standards Board (ASB) prior to being added to the TX35 method. Validation is also important so that these analyte concentrations are accurately reported to better understand the impact these analytes may be having on impairment in DUID casework.

Method Validation Guidelines

According to ANSI/ASB 036, qualitative validation must cover carryover, interference, ion suppression, limit of detection, and processed sample stability. Criteria are set for the limit of detection (LOD), interference, carryover and robustness.[30] Limit of detection is defined as the lowest concentration that produces a response at least three

times greater than the background noise produced by a negative sample. It also must meet predetermined acceptance criteria including retention times and mass spectral ion ratios. The limit of detection is found by running predicted values for LOD in duplicate, in at least three batches. At least 75% of the samples need to meet the previously mentioned criteria to be called the LOD.

Blank matrix samples from at least ten sources should be analyzed to ensure no interferences from the matrices are present. Reference samples should also be analyzed to make sure common analytes do not co-elute from the column, producing interference that could cause misidentification. A target like Δ^8-THC, which is legal, co-eluting with Δ^9-THC can lead to mistakenly reporting a sample positive for Δ^9-THC. Carryover is evaluated by running blank samples between high concentration control samples to determine the highest concentration at which no carryover is observed. Robustness criteria are met by having at least two scientists perform the same analysis and obtain similar results without differences. Once methods are validated to include the new targets, it is important to evaluate the targets' stability.

Cannabinoid Stability

Stability is defined as the resistance an analyte shows to chemical change in a specific matrix at different time points under different conditions.[31] Analytes in a sample are considered stable until the concentration is outside of the accepted bias of the time zero concentration. The accepted bias for an analyte to be considered stable is +/- 20%. Several studies have been conducted to determine the stability of cannabis in oral fluid. Cohier (2017) evaluated Δ^9-THC stability in oral fluid samples collected by two different

oral fluid devices after being stored for 14 days at 4°C. The cannabinoids were stable for the 14 days at 4°C in both devices.[32] Lee (2012) analyzed Δ^9-THC stability at four and 24 weeks at 4°C and -20°C, respectively, and found that Δ^9-THC was stable in OF collected with the Quantisal® device when stored at 4°C up to four weeks and at -20°C up to 24 weeks.[33]

Another factor that affects Δ^9-THC stability in OF is light exposure. Lindholst (2010) showed Δ^9-THC extracted resin degrades via decarboxylation and has a concentration half-life of 35 days in the light and 91 days in the dark.[34] Sannikova (2020) determined that Δ^9-THC in extracted resin is most stable when stored at 4°C with limited light exposure, and that Δ^9-THC loses stability rapidly when stored in warmer conditions under light.[35] Cannabinoid stability can also be affected by the container in which the samples are stored. Djilali (2022) indicated that Δ^9-THC concentration remained stable after 72 hours when the oral fluid sample was stored in a glass vial but was not stable when stored in a polystyrene plastic vial for the same time period.[36] Christophersen (1986) illustrated that Δ^9-THC concentrations in whole blood remain higher in glass vials and the Δ^9-THC blood concentrations for samples stored in plastic tubes dropped between 60 and 100% after storage for 4 weeks.[37]

Little research has been done on the stability of THC-P and THC-O in oral fluid. Maxwell concluded that Δ^9-THC, Δ^{10}-THC, and Δ^8-THC in oral fluid are stable for up to 90 days when stored at 4°C.[38] There has been a fair amount of research showing the stability of Δ^9-THC when stored in blood past 90 days. One study shows that Δ^9-THC is stable in postmortem blood samples when stored at 4°C for six months, but had a concentration decrease greater than 20% in the antemortem blood classifying it as

unstable..[39] Another study showed that Δ^9-THC in antemortem blood was stable for up to four months when stored in glass vials at 4°C and -10°C, but Δ^9-THC concentrations had significantly decreased in samples stored at room temperature after two months.[40] This prior research shows that Δ^9-THC has enhanced stability when stored at cooled or frozen conditions in blood past 90 days of storage. This gives insight on what conditions may provide the best Δ^9-THC stability when stored in oral fluid with Quantisal® buffer for longer periods.

Determining cannabinoid stability and optimum storage conditions is important as it is common for long periods of time to pass between collection of a sample and analysis. Officers may also store oral fluid samples in the trunk of their squad car for a period of time before submitting the evidence to a crime lab. Forensic toxicologists may have to explain the effects of the delay in submission of the evidence on Δ^9-THC concentration. Some samples are retested years after first analysis and any differences in the results need to be able to be explained. Novel cannabinoids are constantly being developed and it is important to understand their stabilities so that samples are preserved in such a way that allows for an accurate interpretation of analysis. Novel cannabinoids have similar structures to Δ^9-THC, but may not have the same stability trends.

OBJECTIVES

1. Validate novel cannabinoids Δ^9-THC-P, Δ^8-THC-P, Δ^9-THC-O, and Δ^8-THC-O using the previously validated cannabinoid oral fluid extraction method
2. Analyze stability of cannabinoids in oral fluid at different time points under different conditions
    a) Stability of Δ^9-THC and novel cannabinoids at room temperature, 4°C, and -20°C up to 90 days
    b) Stability of Δ^9-THC when stored in the light or dark at 4°C
    c) Stability of Δ^9-THC when stored in plastic or glass and separated into aliquots versus samples undergoing multiple freeze/thaw cycles
    d) Δ^9-THC stability in previously analyzed ADFS oral fluid cases after two years of storage
    e) Stability of Δ^9-THC when stored in the trunk of a car (post-collection)
    f) Stability of Quantisal® buffer when stored in trunk of a car (pre-collection)

MATERIALS AND METHODS

Cannabinoid Extraction from Oral Fluid and Analysis Procedure

The standard operating procedure developed by the ADFS was used for the preparation and analysis of all samples. First 500 µL of the oral fluid sample diluted in Quantisal® buffer was pipetted into a labeled 16x125 mm screw cap glass tube. Then 50 µL of an internal standard containing deuterated Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol and cannabinol (0.1 µg/mL) was pipetted into the tube with the oral fluid sample. The screw cap tube was then vortexed, 200 µL of 5% formic acid was added and the tube was vortexed again. Next 3 mL of 80% n-hexane, 10% diethyl ether, and 10% ethyl acetate was added to the sample. The tube was then capped, vortexed, and placed on a rack rotator for five minutes at 40 rpm. The tube was then centrifuged for 10 minutes at 3,000 rpm. The upper phase in the tube was transferred to a labeled 10 mL, glass, conical vial with a glass transfer pipette. Transferring any precipitate or the bottom phase was avoided. The sample in the conical vial was evaporated until completely dry under nitrogen at 45°C. The sample was reconstituted to with 100 µL of 50% mobile phase A and 50% mobile phase B solution and transferred to a labeled auto sampler vial. The auto sampler vial was loaded onto the auto sampler of either the Agilent 6470B, 6470, or 6460 Liquid Chromatography/Triple Quadrupole Mass Spectrometer. Twenty µL of sample was injected onto the instrument with an Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7-micron column. The

instrument was run in binary flow mode with the LC gradient used shown in Table 1. Mobile phase A was 5 mM ammonium formate with 0.1% formic acid in water. Mobile phase B is methanol with 0.1% formic acid. The total run time was 10 minutes with two minutes between injections. A calibration curve was made for every sample set with concentrations at 300, 200, 100, 40, 10, 4, 2, 1, and 0.5 ng/mL for each of the targets listed in Table 2 and positive controls at concentrations of 100, 40, and 10 ng/mL. The negative control was Immunalysis negative synthetic saliva. A blank was run between every sample.

Table 1. *Liquid Chromatography Mobile Phase Gradient*

Time	Mobile Phase A	Mobile Phase B	Flow (mL/min)
0 min	30%	70%	0.5
4 min	20%	80%	0.5
10 min	1%	99%	0.5

Table 2. *Calibration Curve Targets*

Target	Internal Standard	LOD (ng/mL)	Linearity (ng/mL)
Cannabidiol	Cannabinol-d_3	0.2	2.0-300
Cannabigerol	Cannabinol-d_3	0.2	2.0-300
Cannabinol	Cannabinol-d_3	0.5	0.5-300
Δ^9-THC	Δ^9-THC-d_3	1.0	1.0-300
Δ^8-THC	Δ^9-THC-d_3	1.0	*
9R-Δ^{10}-THC	Δ^9-THC-d_3	1.0	*
9S-Δ^{10}-THC	Δ^9-THC-d_3	1.0	*

Δ^9-THC-P	Δ^9-THC-d_3	TBD	TBD
Δ^9-THC-O	Δ^9-THC-d_3	TBD	TBD
Δ^8-THC-P	Δ^9-THC-d_3	TBD	TBD
Δ^8-THC-O	Δ^9-THC-d_3	TBD	TBD
11-hydroxy-Δ^9-THC	11-hydroxy-Δ^9-THC-d_3	4.0	1.0-300
11-nor-9-carboxy-Δ^9-THC	11-nor-9-carboxy-Δ^9-THC-d_3	1.0	2.0-300

*Qualitative only

Validation of Addition of Δ^9-THC-P, Δ^8-THC-P. Δ^9-THC-O, and Δ^8-THC-O to the Existing Cannabinoids in Oral Fluid Method

Limit of detection (LOD) was determined by spiking negative, synthetic, oral fluid samples with Δ^9-THC-P, Δ^8-THC-P. Δ^9-THC-O, and Δ^8-THC-O at concentrations of 10, 4, 2, and 1 ng/mL. Samples were prepared in duplicate at each concentration. This was repeated on six different days at the same concentrations in duplicate. The extraction and analysis procedure were carried out as detailed above.

Matrix interference was evaluated by collecting five oral fluid samples with the Quantisal® and Oral-Eze® oral fluid collection devices from five different volunteers each. Five expectorant oral fluid samples were collected from another five volunteers. The expectorant samples were centrifuged to remove any solid in the sample. All subsequent expectorant oral fluid samples collected were treated in the same way.

Analyte interference was evaluated by spiking oral fluid samples with all previously validated cannabinoids and the novel cannabinoids that were validated. Commonly encountered analytes including benzodiazepines, stimulants, and depressants were spiked into oral fluid and extracted using the same extraction method to ensure

these targets did not interfere with the novel cannabinoids. A full list of all the commonly encountered analytes evaluated can be seen in Appendix B.

Potential Δ^9-THC-P, Δ^8-THC-P. Δ^9-THC-O, and Δ^8-THC-O carryover was analyzed by running blanks after the two highest concentration calibrators during each validation batch analyzed. The extraction and analysis were completed by two different scientists to evaluate robustness. Each ran three of the six total days of testing.

Cannabinoid Stability

Stability at Room Temperature, 4°C, and -20°C

This study replicated the work done by Maxwell to analyze the stability of Δ^9-THC in oral fluid when stored at 20°C (room temperature), 4°C (refrigeration), and -20°C (freezer) for up to 30 days.[38] Samples were stored and analyzed in triplicate at all conditions. An additional study was performed to evaluate the impact of light exposure, storing samples in glass or plastic, and volume of oral fluid sample on Δ^9-THC stability up to 90 days. The collective and individual impacts of these variables on the stability of Δ^9-THC in oral fluid were evaluated.

The stability of Δ^9-THC when stored at 20°C, 4°C and -20°C for up to 30 days was first analyzed. Twenty mL of expectorant oral fluid was collected from two volunteers. Two mL of the expectorant oral fluid was added to 18 (16x100 mm) glass culture tubes. Nine of the tubes were spiked with Δ^9-THC and Δ^8-THC at 200 ng/mL. Nine were spiked with Δ^9-THC and Δ^8-THC at 50 ng/mL. The tubes were then capped and rotated on a rack rotator at 40 rpm for two hours. Quantisal® collection of the oral fluid was simulated by placing one Quantisal® pad into each of the 18 tubes. As soon as

the indicator on the collection pad turned blue, the pads were placed into labeled Quantisal® tubes and capped. The Quantisal® tubes were then rotated on a rack rotator for 4 hours at 40 rpms. The extraction and analysis procedure as outlined above was performed on the 18 samples to establish the initial concentration. The remainder of each sample was transferred to a polystyrene plastic tube. For each of the two concentrations stored at 20°C, 4°C, and -20°C, number of samples analyzed at each time point equals three. Each sample was stored in the dark. Subsequent analyses were performed on each of the 18 samples at 7, 14, and 30 days of storage.

The above experiment was altered and expanded to analyze the stability of Δ^9-THC, Δ^8-THC, Δ^9-THC-P, Δ^9-THC-O, and Δ^8-THC-P at 20°C, 4°C, and -20°C when subjected to different storage variables over 90 days. First, 20 mL of expectorant oral fluid was collected from five different volunteers. Two mL of the expectorant oral fluid was added to 36 (16x100 mm) glass culture tubes. Nine of the tubes were spiked with Δ^9-THC and Δ^8-THC at 200 ng/mL. Nine were spiked with Δ^9-THC and Δ^8-THC at 50 ng/mL. The next nine tubes were spiked with Δ^9-THC-P, Δ^9-THC-O, and Δ^8-THC-P at 200 ng/mL. The final nine tubes were spiked with Δ^9-THC-P, Δ^9-THC-O, and Δ^8-THC-P at 50 ng/mL. The tubes were then capped and rotated on a rack rotator prior to simulated Quantisal® collection as mentioned above. After the time zero extraction, the original samples now contained 2.5 mL of spiked oral fluid. Each of the 36 samples were then aliquoted into five labeled 16x125 mm, glass, screw cap tubes, for a total of 180 0.5 mL aliquots. The aliquots were labeled with the sample number, the storage condition, and time point to be analyzed. These aliquots were then separated into different storage conditions. One hundred and twenty samples were stored in the dark, 60 at 20°C and 60

at -20°. The 60 samples stored at 4°C were stored in the light. The drugs, concentration, and storage temperatures are listed in Table 3. Samples were extracted and analyzed at 7, 14, 30, 60, and 90 days. For both concentrations stored in different conditions, the number of samples analyzed at each time point equals three. Percent change was calculated after the analysis was complete.

Table 3. *Storage Conditions of 0.5 mL Aliquots and Number of Samples in Each Condition at Time Zero*

Spiking Combination	20°C	4°C	-20°C
Δ^9-THC and Δ^8-THC at 200 ng/mL	15 0.5 mL aliquots	15 0.5 mL aliquots	15 0.5 mL aliquots
Δ^9-THC and Δ^8-THC at 50 ng/mL	15 0.5 mL aliquots	15 0.5 mL aliquots	15 0.5 mL aliquots
Δ^9-THC-P, Δ^9-THC-O, and Δ^8-THC-P at 200 ng/mL	15 0.5 mL aliquots	15 0.5 mL aliquots	15 0.5 mL aliquots
Δ^9-THC-P, Δ^9-THC-O, and Δ^8-THC-P at 50 ng/mL	15 0.5 mL aliquots	15 0.5 mL aliquots	15 0.5 mL aliquots

Δ^9-THC Stability when Stored in Light vs Dark Conditions

The stability of Δ^9-THC stored in light vs the dark was evaluated by collecting 10 mL of expectorant oral fluid from two volunteers. Twelve Quantisal® tubes were labeled with concentration and storage conditions. One mL of the expectorant oral fluid was added to each tube. Six of the tubes were spiked with Δ^9-THC at 100 ng/mL and six were with Δ^9-THC at 25 ng/mL and vortexed. Half a milliliter of each sample was extracted to establish the initial concentration of the sample that will be called the time zero concentration. The remaining volume of the samples were then transferred into plastic conical vials. Three samples for each of the two concentrations were stored at 4°C in the

light and the other three samples for each of the two concentrations were stored at 4°C in the dark. Each sample was reanalyzed at 7, 30, and 60 days. The number of samples analyzed at each time for all conditions at both concentrations was three. The concentration percent change was calculated for the 12 samples.

Stability of Δ^9-THC when Stored in Glass vs Plastic Containers

To evaluate the effect of storing samples in plastic vs glass, and whole sample vs aliquoted samples on Δ^9-THC storage stability, 30 samples total were used with 2 sets of 12 and 1 set of 6. First, 20 mL of expectorate oral fluid was collected from 5 different volunteers. One milliliter of expectorant oral fluid each was loaded into 12 different Quantisal® tubes. Six of the tubes were spiked with Δ^9-THC at 100 ng/mL and 6 were spiked with Δ^9-THC at 25 ng/mL. The tubes were vortexed and 0.5 mL of each sample was extracted and analyzed to determine the initial concentration. Three samples for each of the 2 concentrations were transferred to plastic conical vials and stored at 4°C in the dark. Three samples for each of the 2 concentrations were transferred to 16x100 mm glass culture tubes and stored at 4°C in the dark. The samples were reanalyzed at 7, 14, and 30 days to find the concentration percent change. The number of samples analyzed at each time for all conditions at both concentrations was three.

Next, six more glass culture tubes were filled with 2 mL of expectorate oral fluid each and spiked with Δ^9-THC at 100 ng/mL. The extraction and analysis procedure were performed on the 6 samples to establish the initial concentration. Each of the six samples were separated into three 0.5 mL aliquots in different tubes, three samples into plastic conical tubes and three into 16x125 mm glass screw cap tubes. The tubes were labeled

with the original sample and the time point for analysis. The corresponding aliquot tubes for each of the six samples were taken out and analyzed at 7, 14, and 30 days. The number of samples analyzed at each time for all conditions was three.

Two Year Stability of Δ^9-THC Positive Oral Fluid Cases

To determine the two-year stability of Δ^9-THC in oral fluid, 44 ADFS oral fluid DUI samples which were previously analyzed and positive for Δ^9-THC that were first analyzed two years prior were identified. The oral fluid samples' volume was recorded to make sure there was at least 0.5 mL of sample remaining. At the two-year mark after first analysis, oral fluid samples were then extracted and reanalyzed. The current concentration for each sample was compared to the Δ^9-THC concentration reported during the first analysis. Concentration percent change was calculated for the Δ^9-THC concentration by comparing the first analysis Δ^9-THC concentration to the reanalyzed sample concentration.

Δ^9-THC Stability When Stored in the Trunk of a Car (Post-Collection)

To simulate the storage of samples in a trunk of a car during the summer, 10 mL of expectorate oral fluid was collected and centrifuged to remove any solids and 1 ml was added to each of 6 Quantisal® tubes. Three were spiked with Δ^9-THC at 100 ng/mL and three with Δ^9-THC at 50 ng/mL. The Quantisal® tubes were vortexed and 0.5 mL of each sample were extracted and analyzed to establish the time zero concentration. The Quantisal® tubes were stored in the trunk of a car that was in daily use. A digital thermometer that recorded the temperature every 30 minutes was used to monitor the

temperature in the trunk. The samples were reanalyzed at 7 and 30 days and the percentage change concentration calculated for each sample. The number of samples analyzed at each time point for all conditions at both concentrations was three. The experiment was repeated in the fall.

To analyze the amount of time it takes to submit an oral fluid sample to a forensic laboratory after collection, data was compiled to calculate the average time it takes for ADFS to receive a sample after collection. The Laboratory Information Management System (LIMS) was used to document the date of collection for 707 oral fluid samples. The date of receipt by the lab and first analysis was also documented for each oral fluid sample. The number of days between collection and receipt as well as the number of days between receipt and first analysis was calculated for each sample. The mean, median, maximum, and minimum number of days was calculated for each.

Quantisal® Buffer Stability When Stored in Trunk of a Car (Pre-Collection)

To test the suitability of the Quantisal® device collecting Δ^9-THC when the device has been stored in the trunk of a car prior to collection, six Quantisal® tubes were stored in the trunk of a car, room temperature, and 4°C for 1 week. Ten mL of expectorant oral fluid was collected from two different volunteers. All of the Quantisal® tubes were removed from storage and had 1 mL of expectorant oral fluid added to each. One tube from each storage condition was spiked with Δ^9-THC at 25 ng/mL and the other tube from each storage condition was spiked with Δ^9-THC at 100 ng/mL. The Quantisal® samples were used for analysis to compare the concentrations from the different Quantisal® tubes stored in different conditions prior to the addition of oral fluid. The

same process was repeated during the fall. A digital thermometer that recorded the temperature every 30 minutes was used to record the temperature in the trunk. The number of samples analyzed at each concentration for all conditions was one. The total number of samples at all of the experimental conditions for each of the different experiments in this study is shown in Appendix D.

RESULTS AND DISCUSSION

Validation of Δ^9-THC-P, Δ^8-THC-P. Δ^9-THC-O, and Δ^8-THC-O

All novel cannabinoids were qualitatively validated per the ANSI/ASB Standard 036: Standard Practices for Method Validation in Forensic Toxicology.[30] Targets, were not validated quantitatively. The total ion chromatogram (TIC) of the cannabinoids in oral fluid extraction method with the addition of Δ^9-THC-P, Δ^8-THC-P. Δ^9-THC-O, and Δ^8-THC-O at a concentration of 10 ng/mL is shown in Figure 1. The retention times were 7.72 min for Δ^9-THC-P, 7.90 min for Δ^8-THC-P, 7.99 min for Δ^9-THC-O and Δ^8-THC-O. None of the novel cannabinoids co-eluted with any of the previously validated targets. The targets Δ^9-THC-P, Δ^8-THC-P and Δ^9-THC-O were fully resolved. Δ^9-THC-O and Δ^8-THC-O eluted at the same time with a retention time of 7.99 min, but did not interfere with any of the other validated targets or either of the THC-P isomers. Even though Δ^8-THC-P and the THC-O isomers co-eluted, Δ^8-THC-P has a different ion mass to charge ratio allowing for it to be positively identified with no interference. Both isomers of THC-O have the same ion mass to charge ratios so they could not be differentiated. Commonly encountered analytes did not show interference at the concentrations noted in Appendix B.

The new targets Δ^9-THC-P, Δ^8-THC-P, Δ^9-THC-O and Δ^8-THC-O did not interfere with any of the previously validated targets. There was also no interference with any commonly encountered targets with the novel cannabinoids. No changes were

required to the extraction method or instrument parameters to validate these targets. However, Δ^9-THC-O and Δ^8-THC-O co-eluted and could not be distinguished. For this method, Δ^9-THC-O and Δ^8-THC-O will be reported as the non-isomer specific THC-O.

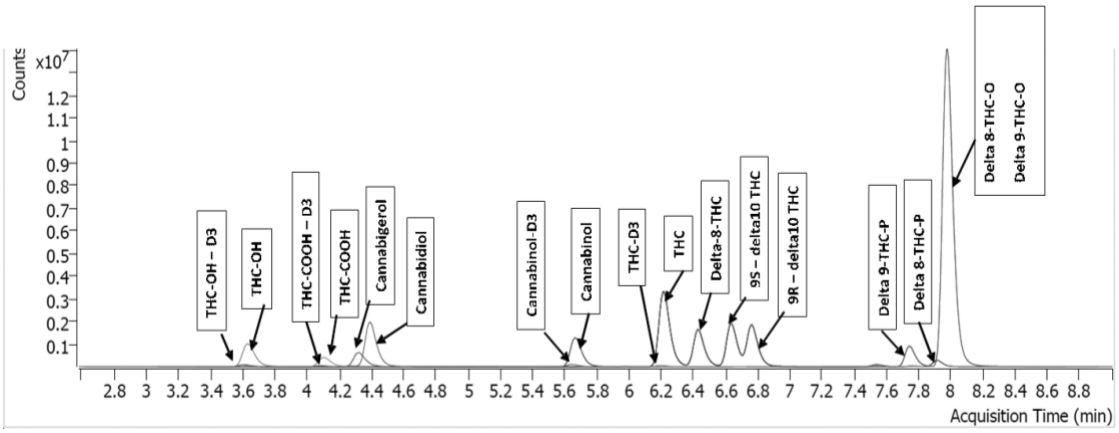

Figure 1. TIC showing all validated targets.

The five Quantisal® blank matrices and the five blank Oral Eze blank matrices were all negative for Δ^9-THC-P, Δ^8-THC-P, Δ^9-THC-O and Δ^8-THC-O. The 10 different blank matrix sources did not show any interference for Δ^9-THC-P, Δ^8-THC-P, Δ^9-THC-O or Δ^8-THC-O. The TIC for one of the blank matrix samples with internal standard is shown in Figure 2.

The Quantisal® and Oral-Eze® blank matrix samples were negative for all targets, meeting the criteria of no matrix interference. The method was proven robust by having two different scientists perform the extraction and analysis. There was also no carryover of any of the new targets when ran at high concentrations. Blanks will be run between case samples due to oral fluid cases routinely having cannabinoid concentrations that exceed the highest calibrator.

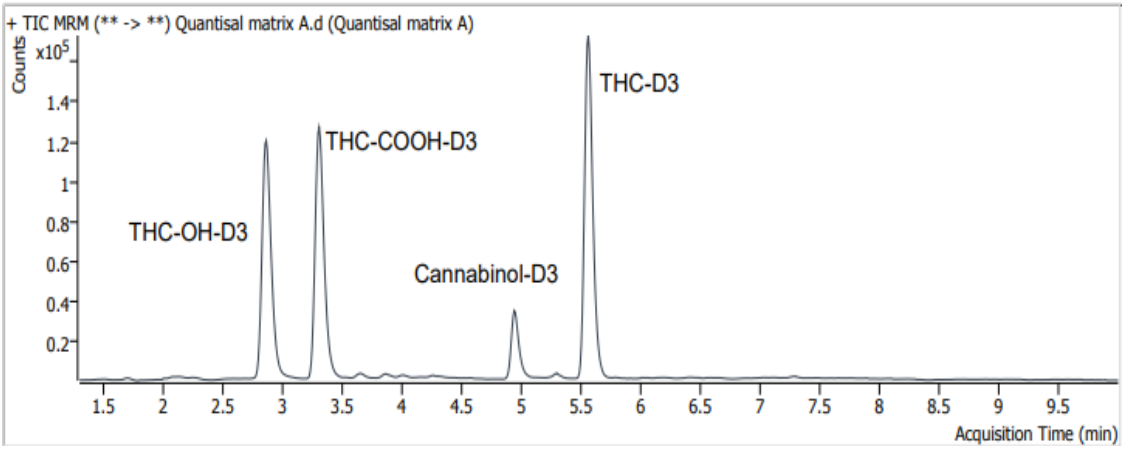

Figure 2. TIC of Quantisal® blank matrix sample with Internal standard.

The limits of detection for Δ^9-THC-P, Δ^9-THC-O and Δ^8-THC-O were 1.0 ng/mL. The limit of detection for Δ^8-THC-P was determined to be 2.0 ng/mL. The TIC with Δ^9-THC-P and Δ^9-THC-O at their LOD of 1 ng/mL is shown in Figure 3. The TIC with Δ^8-THC-O at its LOD of 1 ng/mL is shown in Figure 4. The TIC with Δ^8-THC-P at its LOD of 2 ng/mL is shown in Figure 4 The lower and upper limits of quantitation (LLOQ and ULOQ) were not determined for the new cannabinoids as they are not yet controlled. The limit of detection and range of concentrations for quantitation for the cannabinoids method that includes the new targets being validated are shown in Table 4.

The LOD criteria was passed at 1 ng/mL in at least 75% of the LOD samples for Δ9-THC-P, Δ9-THC-O, and Δ8-THC-O. The LOD criteria did not pass at 1 ng/ml for Δ8-THC-, but it did pass in 75% of samples at 2 ng/mL. The median concentration for Δ8-THC and Δ9-THC in 554 oral fluid ADFS cases was 21 ng/mL and 31 ng/mL respectively. While THC-O and THC-P have not been detected in casework, LODs of 1 or 2 ng/mL will be sufficient for laboratories to detect the target cannabinoids in casework even at low concentrations. This study qualitatively validated the addition of

Δ^9-THC-P, Δ^8-THC-P, Δ^9-THC-O, and Δ^8-THC-O to the cannabinoids in oral fluid extraction method at ADFS.

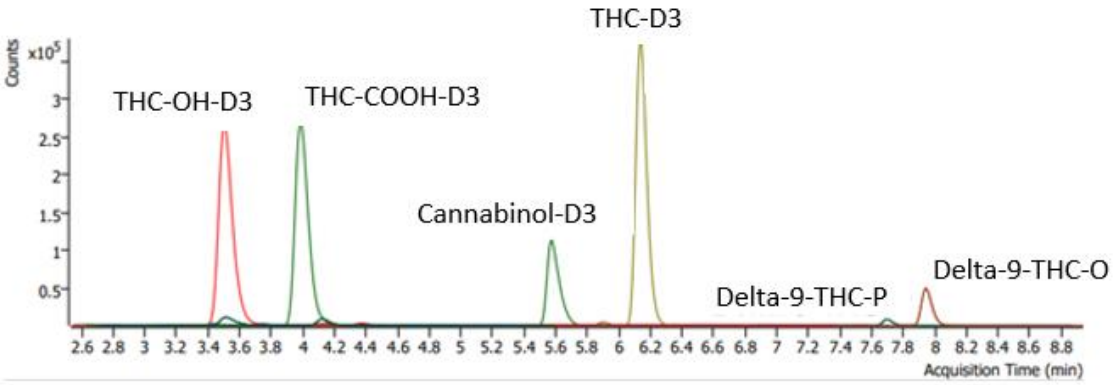

Figure 3. TIC of Δ^9-THC-P and Δ^9-THC-O at LOD of 1 ng/mL

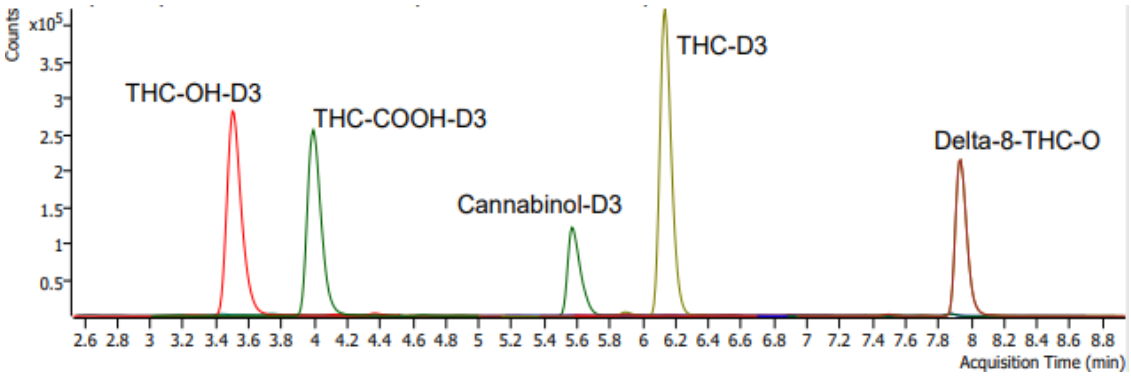

Figure 4. TIC of Δ^8-THC-O at LOD of 1 ng/mL

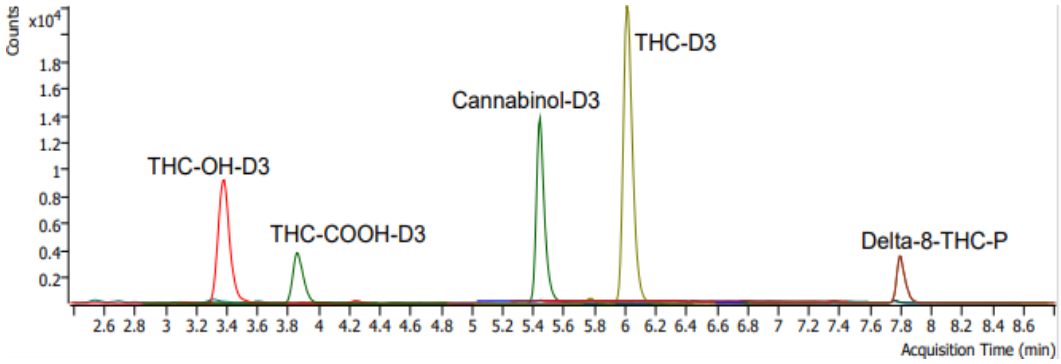

Figure 5. TIC of Δ^8-THC-P at LOD of 2 ng/mL

Table 4. *Limit of Detection of Newly Validated Targets*

Target	Internal Standard	LOD (ng/mL)	Linearity (ng/mL)
Cannabidiol	Cannabinol-d_3	0.2	2.0-300
Cannabigerol	Cannabinol-d_3	0.2	2.0-300
Cannabinol	Cannabinol-d_3	0.5	0.5-300
Δ^9-THC	Δ^9-THC-d_3	1.0	1.0-300
Δ^8-THC	Δ^9-THC-d_3	1.0	*
9R-Δ^{10}-THC	Δ^9-THC-d_3	1.0	*
9S-Δ^{10}-THC	Δ^9-THC-d_3	1.0	*
Δ^9-THC-P**	Δ^9-THC-d_3	1.0	*
Δ^9-THC-O**	Δ^9-THC-d_3	1.0	*
Δ^8-THC-P**	Δ^9-THC-d_3	2.0	*
Δ^8-THC-O**	Δ^9-THC-d_3	1.0	*
11-hydroxy-Δ^9-THC	11-hydroxy-Δ^9-THC-d_3	4.0	1.0-300
11-nor-9-carboxy-Δ^9-THC	11-nor-9-carboxy-Δ^9-THC-d_3	1.0	2.0-300

**Validated as part of this study *Qualitative only

Cannabinoid Stability in Oral Fluid

Replicated 30-Day Stability at Room Temperature, 4°C, and -20°C as whole samples

For the first stability experiment, 18 oral fluid samples were spiked with Δ^9-THC at concentrations of 200 ng/mL in nine samples and 50 ng/mL in the other nine samples.

Oral fluid samples were stored as whole samples, not aliquoted, and the same tube was reaccessioned from four times for the analysis at 0, 7, 14, and 30 days. These samples were stored in plastic tubes in darkness and at 20°C, 4°C, or -20°C for 30 days. The stability of samples spiked with Δ^9-THC at 200 ng/mL is shown in Figure 6. The average initial concentrations for samples stored at 20°C, 4°C, and -20°C were 103, 94, and 91 ng/mL respectively. This was approximately 50% of the concentrations that the samples were spiked at and recoveries were consistent with previous studies performed by Maxwell and Lee. The initial Δ^9-THC concentration for each of the samples in this study can be seen in Appendix C. Samples were expected to yield nearly a 50% recovery, because of this, samples were spiked at 200 ng/mL and 50 ng/mL to target initial concentrations of 100 and 25 ng/mL. Samples spiked at high concentrations had average concentration percent changes of -2.2%, +4.0%, and +2.3% when stored for 30 days at 20°C, 4°C, and -20°C, respectively. All sample concentrations at 30 days were within 20% of their respective initial concentrations for all three conditions, demonstrating good stability.

The stability of samples spiked at 50 ng/mL of Δ^9-THC when stored in the dark in plastic tubes at 20°C, 4°C, and -20°C is shown in Figure 7. The average initial concentrations for the samples spiked at 50 ng/mL was 26, 22, and 22 ng/mL for the 20°C, 4°C, -20°C samples respectively. Similar to the high concentration condition, the recovery was approximately 50% the spiked concentrations. The average concentration percent change for samples stored at 20°C, 4°C, and -20°C after one month of storage was +0.4%, -6.8%, and -0.9% respectively. All sample concentrations after 30 days of storage, regardless of storage conditions, were within 20% of the initial concentrations.

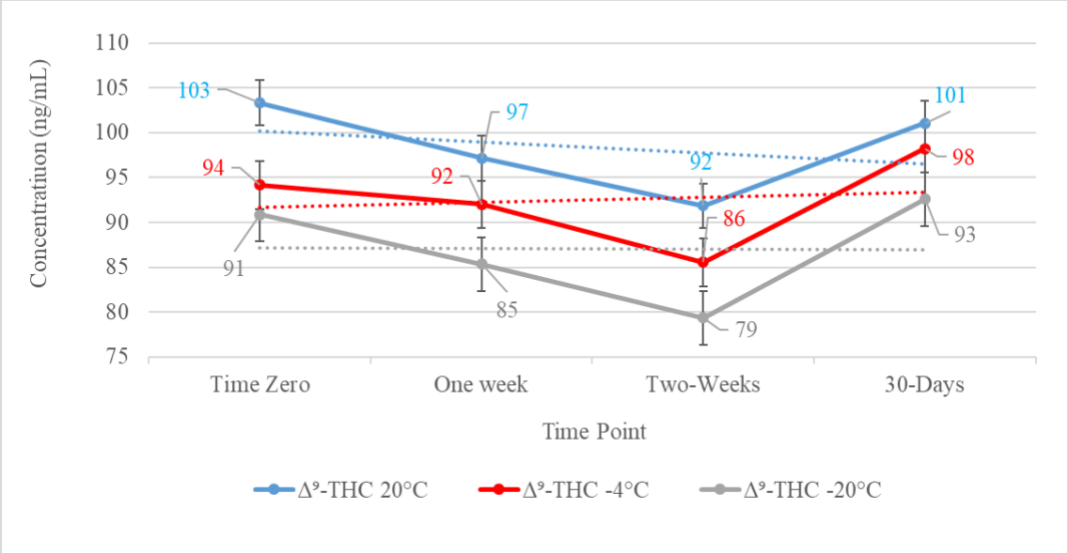

Figure 6. Δ^9-THC stability in oral fluid spiked at concentrations of 200 ng/mL stored at room temperature, 4°C, and -20°C from 0-30 days

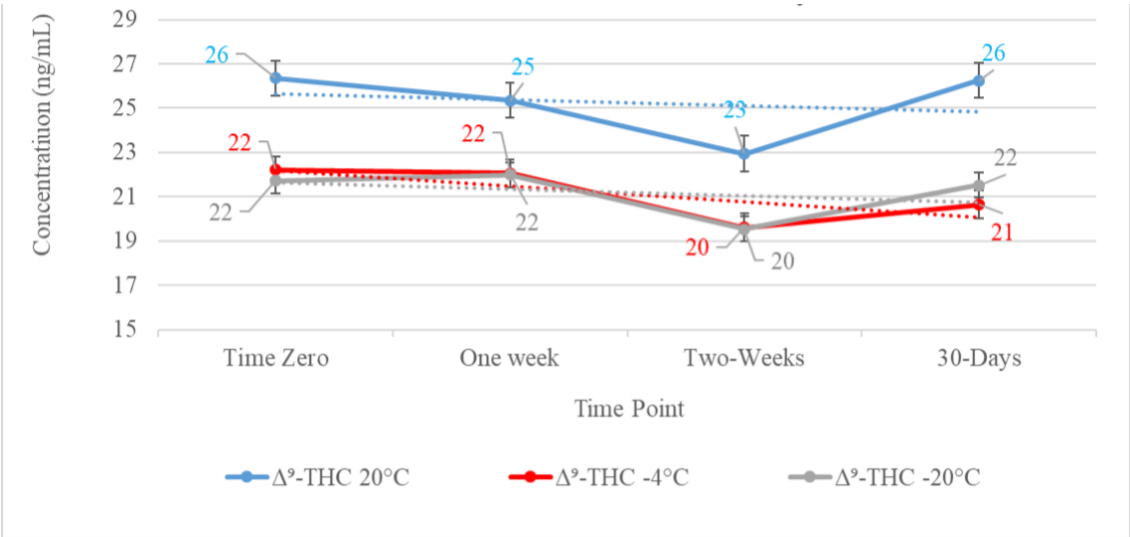

Figure 7. Δ^9-THC stability in oral fluid spiked at concentrations of 50 ng/mL stored at room temperature, 4°C, and -20°C from 0-30 days

The first stability experiment examining the stability of Δ^9-THC up to one month of storage at 20°C, 4°C, -20°C was designed to reproduce results from the Maxwell study that concluded Δ^9-THC is most stable at 4°C when stored for 90 days.[38] Similar to Maxwell, this study stored samples in the dark and in plastic tubes at all conditions, but used a sample size of three instead of two per each sample set. Samples were also stored as a whole, not aliquoted, and the same sample tube was reaccessioned from for the analysis at 0, 7, 14, and 30 days. After one month, samples at all conditions remained stable. The reanalyzed concentrations were within 20% of the original concentrations. There was a small average concentration increase for samples spiked at high Δ^9-THC concentrations when stored at 4°C and -20°C for one month. The concentration increases did not exceed the uncertainty of measurement for this method which was 18%. This study is consistent with other studies performed by Maxwell, Lee, and Cohier showing that Δ^9-THC is stable at both high and low concentrations when stored at 4°C and -20°C for one month.[32, 33, 38] The data is inconsistent with the Maxwell study as this data shows Δ^9-THC is stable for up to a month when stored at room temperature, whereas Δ^9-THC was unstable at two weeks in the Maxwell study.[38] This adds to the literature showing Δ^9-THC is stable in oral fluid for up to a month when stored in the dark, in plastic tubes, and as a non-aliquoted sample when stored at 4°C or -20°C.

Collective Impact on Cannabinoid Stability for Aliquoted Samples Stored in the Light or Dark at 20°C, 4°C, and -20°C Up to 90 Days

The above stability experiment was altered to investigate 18 samples spiked at concentrations of 50 ng/mL (n=9) or 200 (n=9) ng/mL and another 18 samples spiked at

concentrations of 50 (n=9) ng/mL or 200 ng/mL (n=9). These samples were pre-aliquoted into 0.5 mL portions for single analysis from each tube. Samples were stored in plastic tubes at 20°C in the dark, 4°C in partial light, or -20°C in the dark for 0-90 days. The stability of Δ^9-THC spiked at 200 ng/mL (high concentration) and stored at room temperature, in the cooler, or in the freezer for this experiment is shown in Figure 8. The Δ^9-THC concentration in room temperature samples decreased by 25% after storage for one week. After 90 days, the concentrations decreased by 60%. Storage at 4°C exposed to partial light decreased the Δ^9-THC concentration by 28% and by 69% after one week and 90 days, respectively. The samples stored in the dark and at -20°C had an average decrease in Δ^9-THC of 40% after one week and 52% after 90 days of storage. The concentration of Δ^9-THC stored at 4°C did not significantly change between 7-14 days and 60-90 days.

The stability of Δ^9-THC spiked at 50 ng/mL (low concentration) and stored at room temperature, in the cooler, and in the freezer for this experiment is shown in Figure 9. After one week of storage the Δ^9-THC concentration decreased by 39%, 35%, and 33% for samples stored at room temperature, in the cooler, and in the freezer, respectively. The Δ^9-THC concentration for oral fluid samples stored at room temperature, in the cooler, and in the freezer decreased by 65%, 69%, and 47% after 90 days of storage, respectively. The Δ^9-THC concentrations under all three storage conditions did not significantly change between 7-14 days.

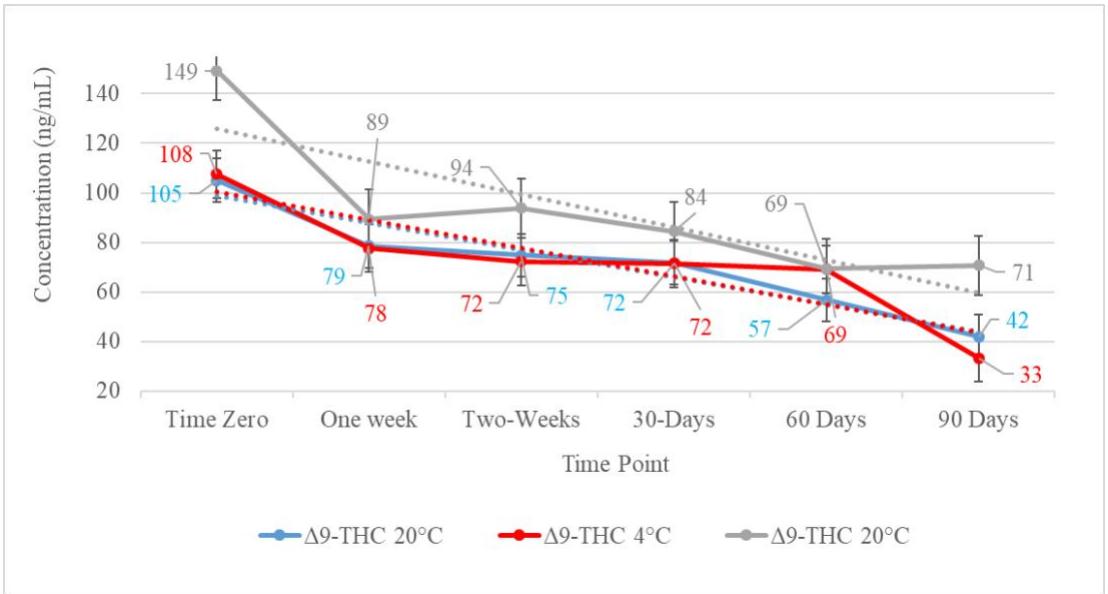

Figure 8. Δ^9-THC stability in oral fluid spiked at concentrations of 200 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

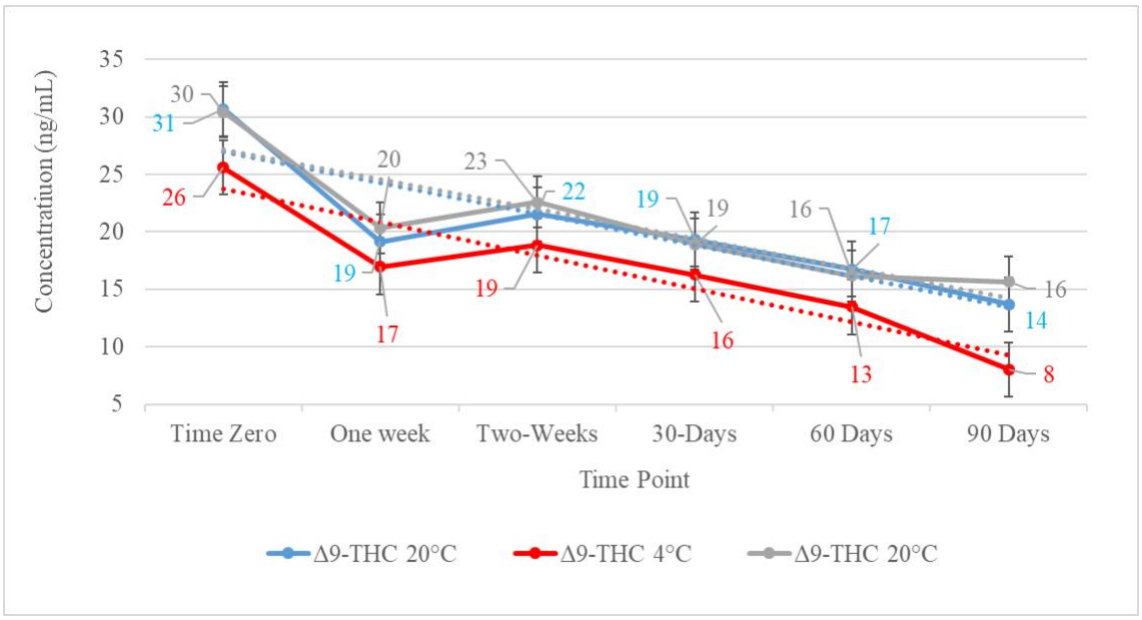

Figure 9. Δ^9-THC stability in oral fluid at concentrations of 50 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

Figure 10 shows the stability of Δ^8-THC in oral fluid spiked at high concentrations when stored in room temperature, the cooler, or in the freezer. After one week of storage at room temperature, in the cooler, and in the freezer the average Δ^8-THC concentration remained stable at room temperature and in the cooler, but was unstable in the freezer. Following 90 days of storage at room temperature, in the cooler, and in the freezer, the average Δ^8-THC concentration decreased by 24%, 55%, and 51% respectively.

The stability of Δ^8-THC spiked at low concentrations in oral fluid stored at room temperature, in the cooler, and in the freezer is shown in Figure 11. The average Δ^8-THC concentrations remained stable at all conditions. Average concentrations decreased by 24%, 58%, and 15% respectively after 90 days.

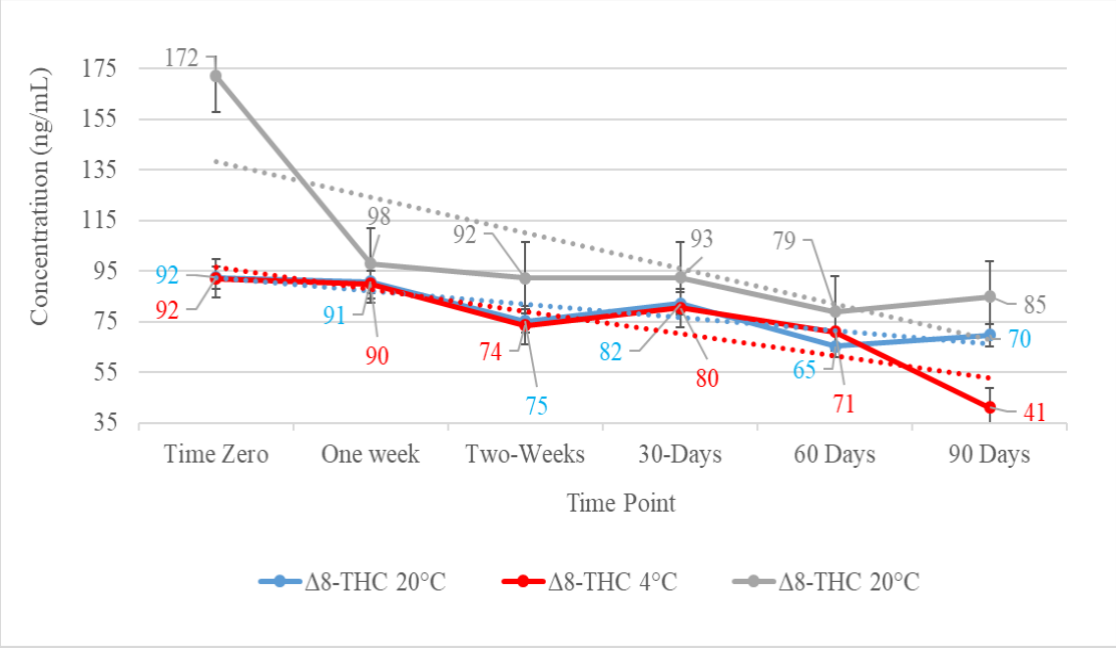

Figure 10. Δ^8-THC stability in oral fluid spiked at concentrations of 200 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

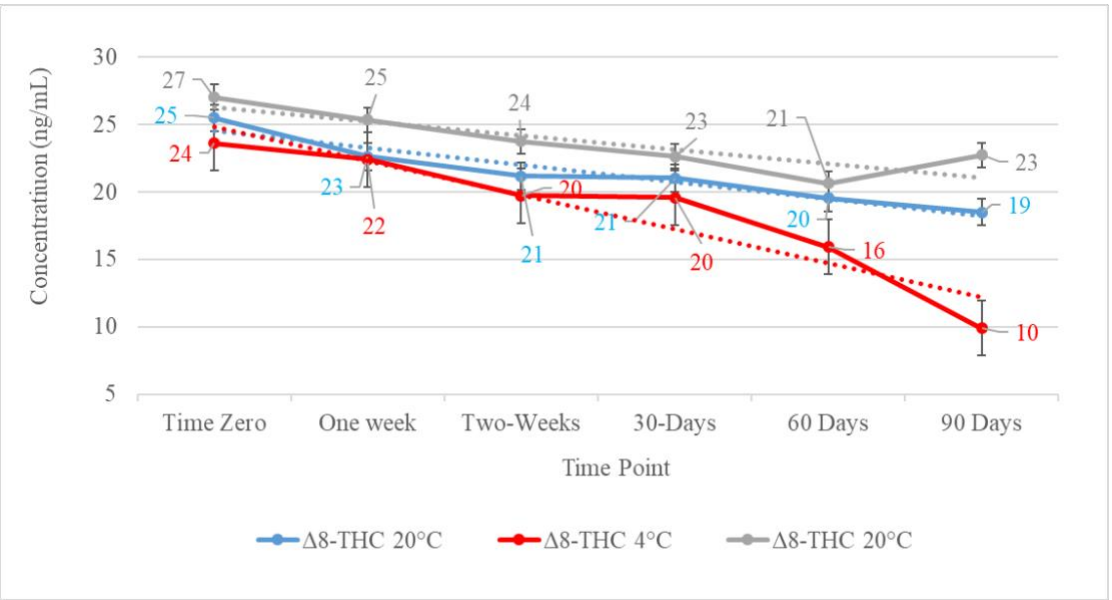

Figure 11. Δ^8-THC stability in oral fluid spiked at concentrations of 50 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

The stability of THC-O in oral fluid at high concentrations stored at room temperature, in the cooler, and in the freezer for 0-90 days is shown below in Figure 12. The average concentration of THC-O decreased by 40%, 29%, and 50% following one week and by 93%, 73%, and 16% after 90 days for samples stored at room temperature, in the cooler, and in the freezer respectively.

The stability of THC-O spiked at low concentrations stored at room temperature, in the cooler, and in the freezer for 0-90 days is shown in Figure 13. The average concentration of THC-O in oral fluid decreased by 45%, 25%, and 30% after one week and by 100% and 50% for samples stored at room temperature and in the cooler respectively after 90 days. The THC-O concentration increased by 10% after 90 days of storage for samples stored in the freezer. Concentrations of THC-O increased more than

18% between one week and two weeks at all conditions. There was also average concentration increases greater than 18% for samples stored at -20°C and 4°C.

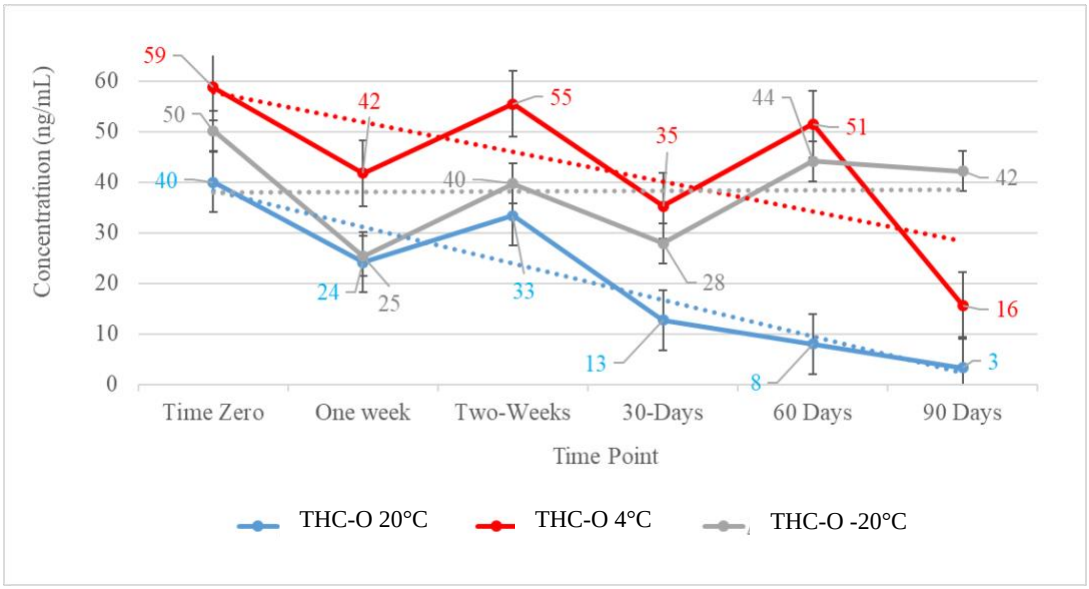

Figure 12. THC-O stability in oral fluid spiked at concentrations of 200 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

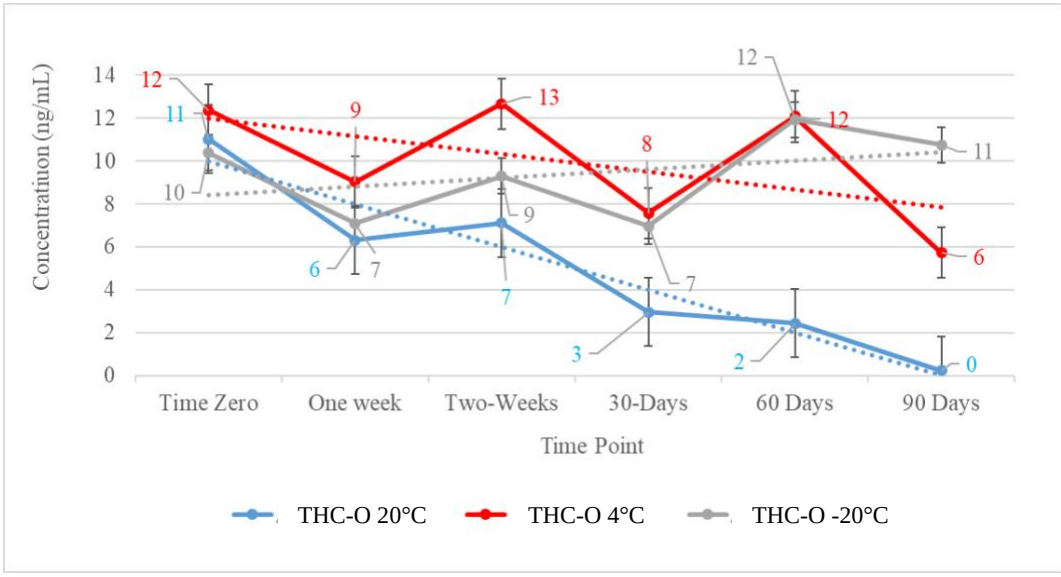

Figure 13. THC-O stability in oral fluid at spiked at concentrations of 50 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

The stability of Δ^9-THC-P in oral fluid at high concentrations stored at room temperature, in the cooler, and in the freezer for 0-90 days is shown below in Figure 14. The average concentration of Δ^9-THC-P remained stable at room temperature and in the cooler, but was unstable in the freezer following one week. Average concentrations decreased by 91%, 72%, and 47% after 90 days for samples stored at room temperature, in the cooler, and in the freezer respectively.

The stability of Δ^9-THC-P spiked at low concentrations stored at room temperature, in the cooler, and in the freezer for 0-90 days is shown in Figure 15. The average concentration of Δ^9-THC-P in oral fluid decreased by 19%, 26%, and 44% after one week and by 52%, 68%, and 33% following 90 days of storage at room temperature, in the cooler, and in the freezer respectively.

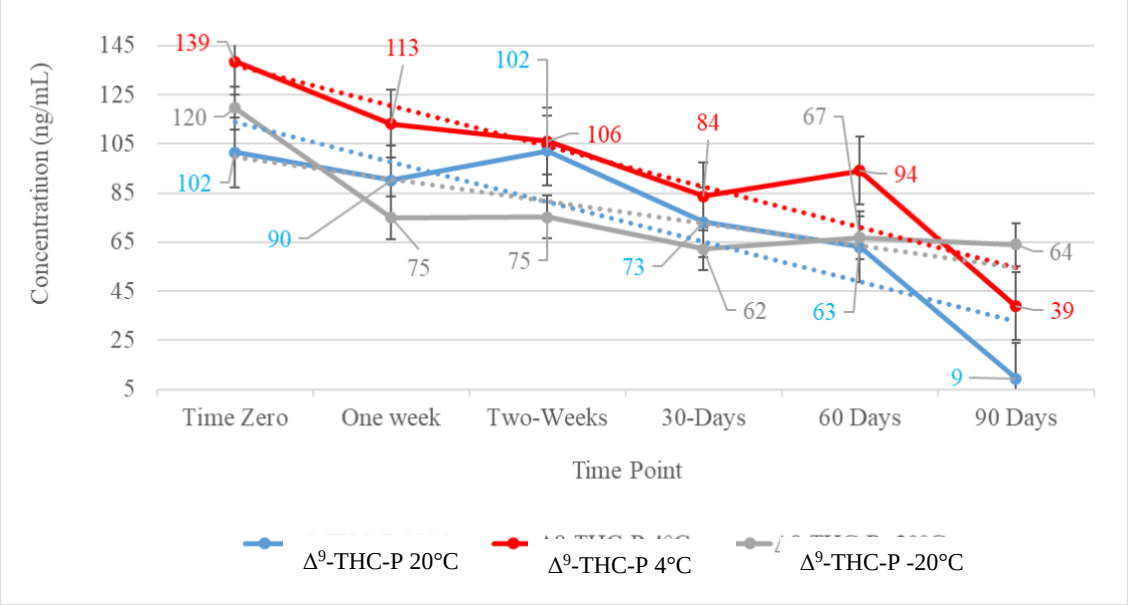

Figure 14. Δ^9-THC-P stability in oral fluid spiked at concentrations of 200 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

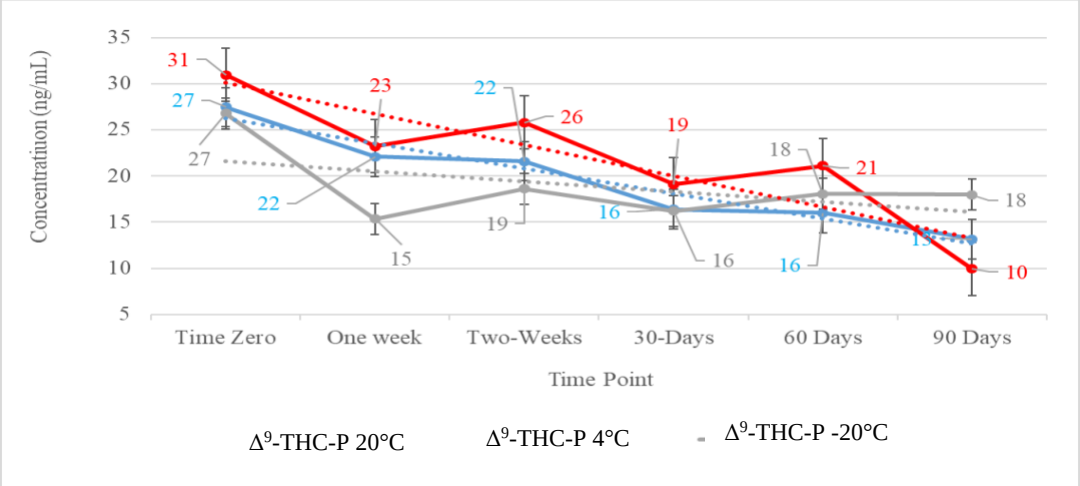

Figure 15. Δ^9-THC-P stability in oral fluid spiked at concentrations of 50 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

The stability of Δ^8-THC-P in oral fluid at high concentrations stored at room temperature, in the cooler, and in the freezer for 0-90 days is shown below in Figure 16. The average concentration of Δ^8-THC-P decreased by 21%, 29%, and 43% following one week and decreased by 79%, 64%, and 31% from 0-90 days for samples stored at room temperature, in the cooler, and in the freezer respectively.

The stability of Δ^8-THC-P spiked at low concentrations stored at room temperature, in the cooler, and in the freezer for 0-90 days is shown in Figure 17. The average concentration of Δ^8-THC-P in oral fluid decreased by 13%, 22%, and 26% after one week and by 52%, 59%, and 17% from 0-90 days of storage at room temperature, in the cooler, and in the freezer respectively.

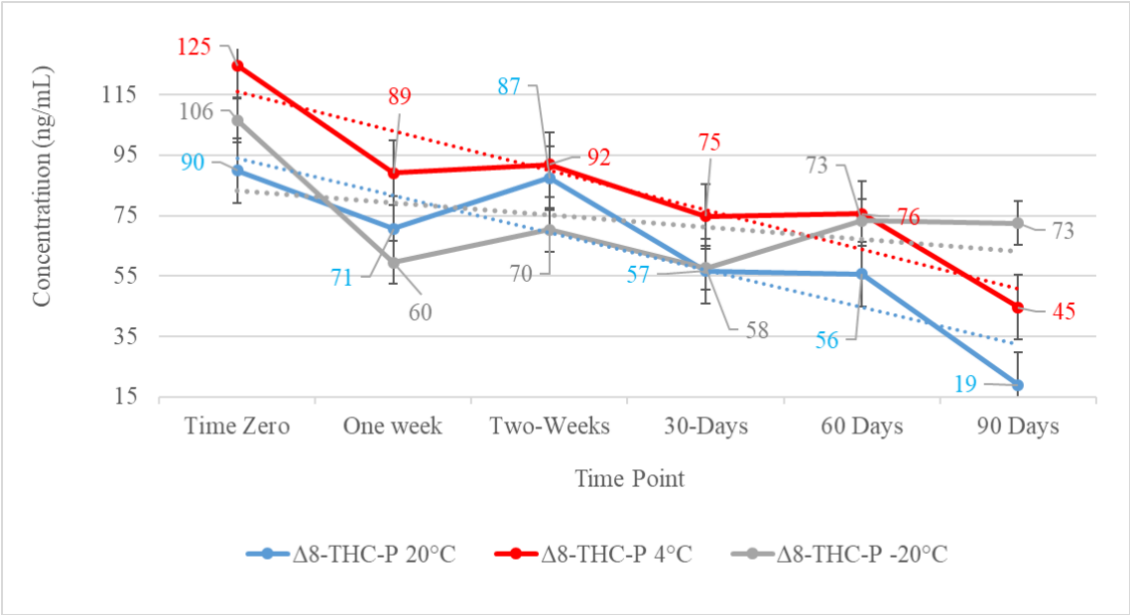

Figure 16. Δ^8-THC-P stability in oral fluid spiked at concentrations of 200 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

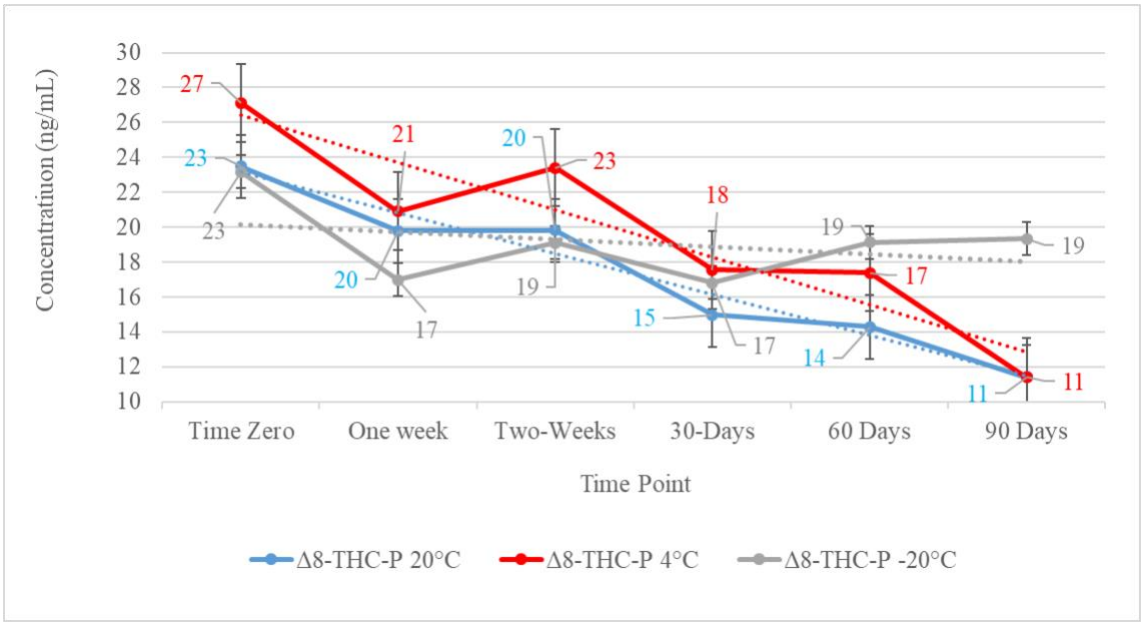

Figure 17. Δ^8-THC-P stability in oral fluid spiked at concentrations of 50 ng/mL stored at room temperature, 4°C, and -20°C from 0-90 days in aliquoted stability study

It is important to evaluate the stability of Δ^9-THC in different storage conditions in order determine the length of time Δ^9-THC can be. If Δ^9-THC is unstable, then the reported concentration may be much lower than the concentration of Δ^9-THC at the time of collection. The potential for measuring low Δ^9-THC concentrations due to instability in the time before analysis may impact testimony related to drug effects during a DUI trial. However, decreased concentration or lack of detection would benefit the defendant.

Although novel cannabinoids such as THC-O and THC-P are not quantitated, is important to understand how different storage conditions affect their stability in oral fluid. The changing legislature surrounding synthetic novel cannabinoids suggests that the novel cannabinoids may require quantitation in the future.

The stability study where samples were aliquoted prior to storage at the three storage conditions examined how the stability of cannabinoids may be affected by changing different storage variables. The variables changed for the study were the light conditions and samples were aliquoted. All samples were aliquoted for this stability experiment. Samples stored at 20°C and -20°C were kept in the dark whereas samples at 4°C were stored in the light. After one week, Δ^9-THC samples were unstable in all storage conditions with concentration decreases greater than 20%. After 90 days of storage all samples had lost 50-60% of the Δ^9-THC. Samples spiked with Δ^9-THC had enhanced stability when stored in the freezer for 90 days compared to samples stored at room temperature and in the cooler. The samples stored at 4°C were the least stable which is not consistent with previous studies. Maxwell analyzed samples stored under the same conditions, except that samples were all stored in the dark and the samples were not aliquoted. Under those conditions, Δ^9-THC was stable for up to 90 days when stored at

4°C and up to 60 days at -20°C.[38] Lee showed that Δ^9-THC in oral fluid was stable for four weeks when stored at 4°C and was not stable after four weeks when stored at -20°C.[33] Scheidweiler recommends storing oral fluid samples at 4°C.[41] Anizan et al. shows that Δ^9-THC was stable in 4°C for up to four weeks before becoming unstable and Δ^9-THC was most stable in oral fluid when stored at -20°C for 24 weeks.[42] The Δ^9-THC instability at 4°C and -20°C in this study may be due to splitting the sample into aliquots or due to the different light conditions. Oral fluid samples of lower volume that are of the same drug concentration as samples stored in higher volumes which are stored in the same size tube have a greater surface area of drug that is exposed to the storage tube material. Δ^9-THC could be sticking to the sides of the tube more in lower volumes than in higher volumes due to the increased surface area contact. The Δ^9-THC sticking to the tube contributed to the differences in stability between the first 30 day experiment and the aliquoted stability experiment.

In high concentrations Δ^8-THC was stable in oral fluid samples stored at room temperature and in the cooler. The samples were not stable after one week when stored in the freezer undergoing a 43% decrease in concentration. High concentration Δ^8-THC samples stored at room temperature and in the cooler remained stable for 30 days. Beyond 30 days, samples stored at all conditions were unstable with samples stored at 4°C being the least stable. Low Δ^8-THC concentration samples were stable at all conditions after one week. The samples remained stable for 30 days in samples when stored at room temperature and in the cooler. After 90 days, low concentration Δ^8-THC samples remained stable when stored in the freezer The samples stored in the cooler had the largest concentration decrease. This is inconsistent with Maxwell's study that shows

Δ^8-THC is most stable in oral fluid when stored at 4°C at high concentrations, but it does also show Δ^8-THC is stable up to 90 days in low concentrations when stored at -20°C.

THC-O was unstable after one week under all conditions, but the concentration increased between 1-2 weeks. The concentrations decreased at 30 days and again THC-O concentrations increased at 60 days in samples stored at 4°C and -20°C. The total THC-O concentration decrease from 0-90 days for samples stored in the freezer was 16% which classifies it as stable since it is less than 20%. THC-O samples stored at room temperature and in the cooler were unstable after 90 days. The increase in THC-O concentration could be caused by cannabinoid conversion into THC-O since it was spiked in samples with both isomers of THC-P. More research needs to be done to consider if any cannabinoids convert into THC-O over time.

Δ^8-THC-P also had an increase in concentration at the 60-day mark in samples stored at -20°C. Δ^8-THC-P was unstable in the freezer after one week, but only had a decrease of 17% from 0-90 days in samples spiked at low concentrations to classify it as stable. Δ^8-THC-P was stable at high concentrations up to two weeks when stored at room temperature. At high concentration, Δ^9-THC-P samples remained stable for two weeks when stored at room temperature and at 4°C. In low concentrations of Δ^9-THC-P, the target drug was stable for two weeks when stored at room temperature. All samples were unstable after 90 days, but Δ^9-THC-P had the lowest overall concentration decrease in samples stored at 4°C. Many novel cannabinoids show a dramatic decrease in concentration for the samples stored in the freezer after one week which could be a result of the samples being split to avoid the freeze/thaw cycle as explored later. More research

needs to be done to understand the stability of novel cannabinoids for extended periods of storage at different time points.

Δ^9-THC Stability in Light vs Dark

To determine the impact of exposure to light prior to testing, 12 samples were spiked with Δ^9-THC at concentrations of 100 or 25 ng/mL and stored at 4°C in the light or dark for 0-60 days. Three samples were spiked at 100 ng/mL and stored in the dark and three were spiked at 100 ng/mL and stored in the light. Three samples were spiked at 25 ng/mL and stored in the dark and three were spiked at 25 ng/mL and stored in the light. The stability of Δ^9-THC in oral fluid at low concentrations stored in the cooler in either light or dark environments is shown in Figure 18. The concentration did not change after one week of storage for either the samples stored in the light or in the dark. The Δ^9-THC concentration did not decrease after two months of storage in the dark in the cooler, however, the concentration decreased by 17% for samples stored in the light.

The stability of Δ^9-THC in oral fluid at high concentrations stored in the cooler in either light or dark environments is shown in Figure 19. The average Δ^9-THC concentration for samples stored in the dark did not change after two months. After one week the average Δ^9-THC concentration for samples stored in the light decreased by 3.3% and by 17% after two months of storage.

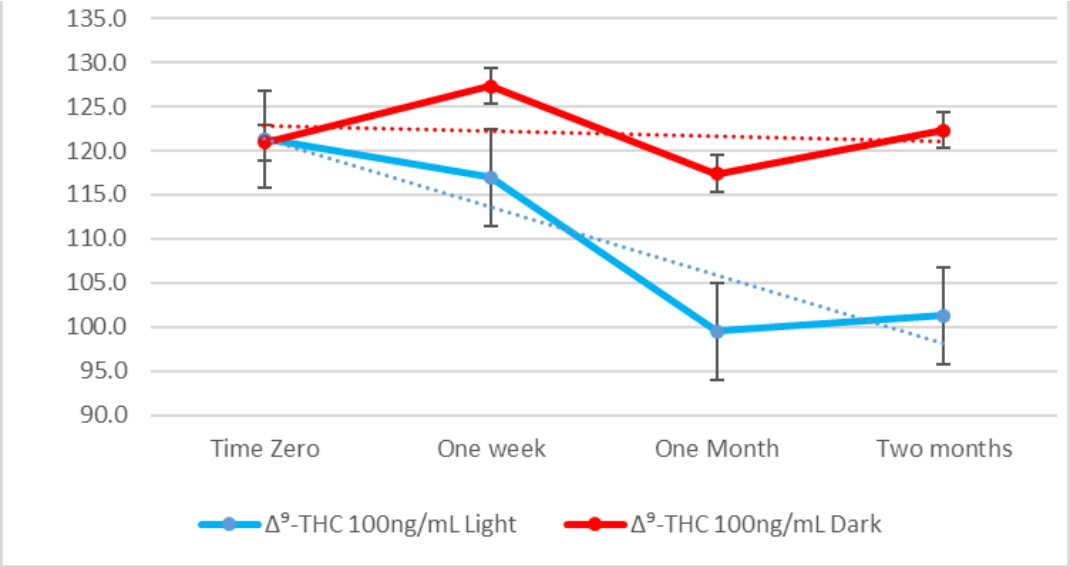

Figure 18. Stability of Δ^9-THC in oral fluid spiked at concentrations of 25 ng/mL when stored in the light vs the dark at 4°C

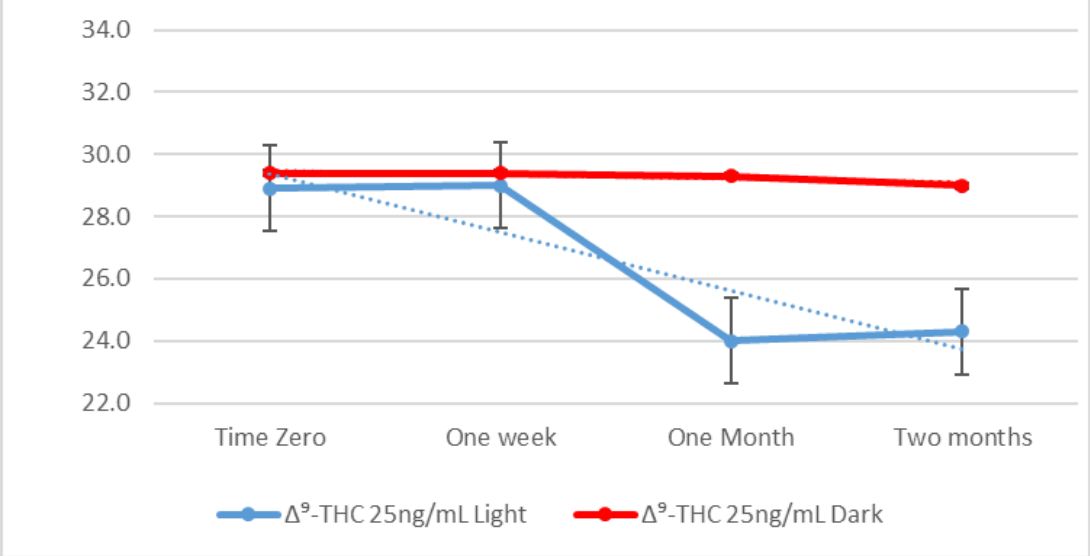

Figure 19. Stability of Δ9-THC in oral fluid spiked at concentrations of 100 ng/mL when stored in the light vs the dark at 4°C

The effects of light on stability of Δ^9-THC have been well documented to show that light decreases Δ^9-THC stability.[34, 35] This study adds to the literature about how Δ^9-

THC stability may be affected by the light conditions in which samples are stored. We hypothesize that Δ^9-THC stability was negatively impacted by light exposure during the 90-day aliquoted stability study. This would explain the differences in stability when compared to other studies.

This study is consistent with other light vs dark studies showing that Δ^9-THC is more stable in the dark and has decreased stability when stored in the light. Since the 0-90 day aliquoted stability samples stored in the cooler were exposed to light, this explains part of the reason why the Δ^9-THC in oral fluid stability at 4°C was not enhanced compared to samples stored in room temperature like most studies suggest. The samples at room temperature in the 0-90 day study were stored in the dark which made them more stable than if they were stored in the light. The light in the cooler had negative effects on Δ^9-THC stability that was a contributing factor as to why Δ^9-THC in oral fluid concentration decreases were much higher after one week compared to other stability studies.

Stability of Δ^9-THC *Stored in Glass vs Plastic Containers*

Samples were spiked with Δ^9-THC at concentrations of 100 (high conc.) or 25 (low conc.) ng/mL before being split into glass or plastic tubes as whole (non-aliquoted) or aliquoted samples. Three samples were spiked at 100 ng/mL and stored as a whole sample in plastic and three samples were spiked at 25 ng/mL were stored the same way. Three samples were spiked at 100 ng/mL and stored as a whole sample in glass and three samples were spiked at 25 ng/mL and stored in glass. Three samples were spiked at 100 ng/mL, aliquoted, and stored in plastic. Three samples were spiked at 100 ng/mL,

aliquoted, and stored in glass. The samples were then stored at 4°C for 0-30 days. The Δ^9-THC concentrations at zero days to one month for oral fluid samples spiked at high concentrations stored in the cooler in either plastic or glass tubes is shown in Figure 20. Following one month of storage, Δ^9-THC samples stored in plastic and glass remained stable.

The stability of Δ^9-THC in oral fluid samples spiked at low concentrations stored in the cooler in either plastic or glass tubes is illustrated in Figure 21. The average Δ^9-THC concentration for samples stored in plastic tubes remained stable after one month. For the samples stored in glass tubes Δ^9-THC concentrations remained stable one month of storage, respectively.

Figure 22 shows the samples spiked at high concentrations stored in plastic or glass (non-aliquoted) compared to samples that had 0.5 mL aliquoted into separate plastic or glass tubes. Low concentrations were not assessed in this study. The aliquoted samples stored in plastic tubes had an initial average Δ^9-THC concentration of 116 ng/mL remained stable after one week of storage and decreased by 22% after one month. The aliquoted samples stored in glass tubes had an average initial concentration of 102 ng/mL. The average concentration remained stable after one week and unstable after one month.

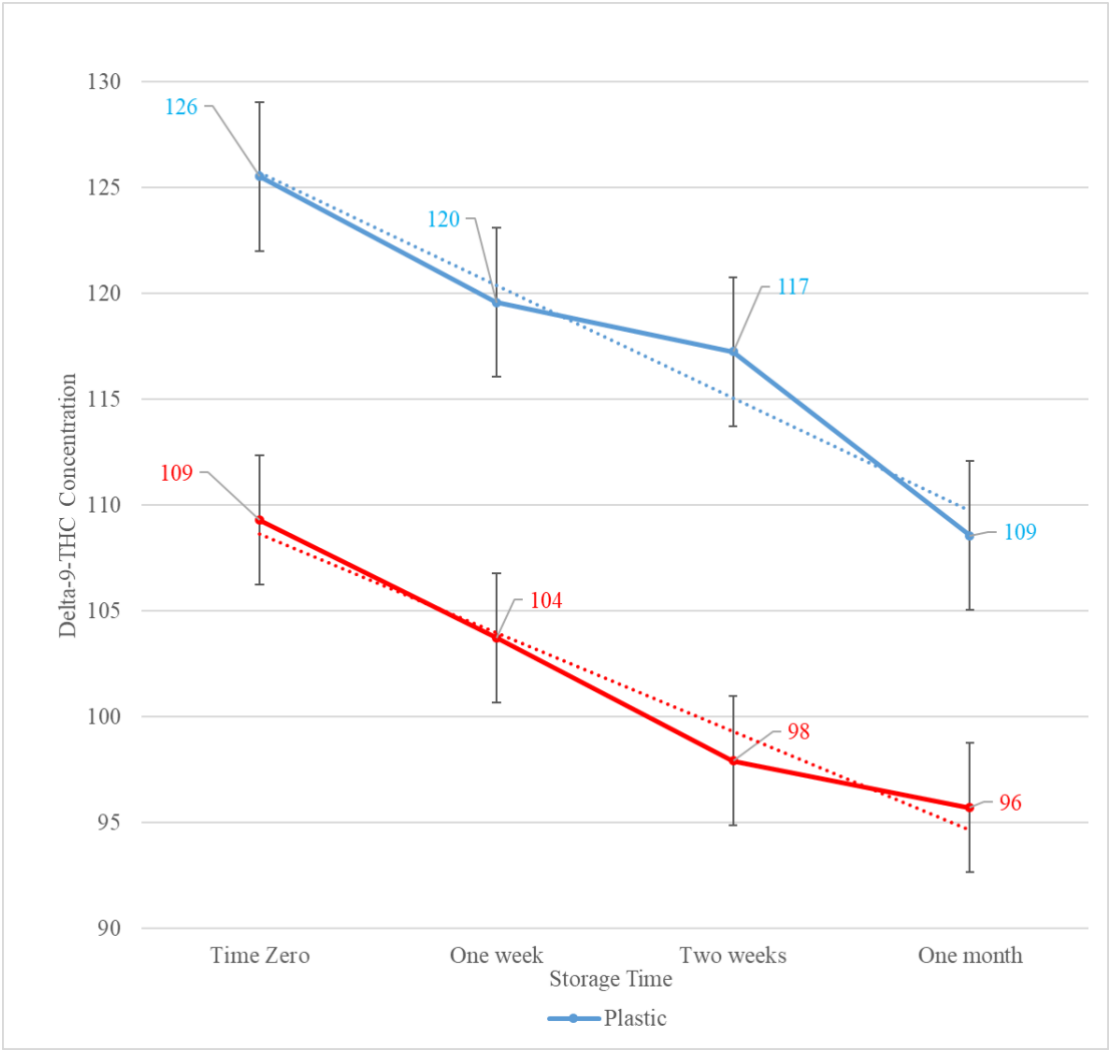

Figure 20. Stability of Δ^9-THC in oral fluid spiked at concentrations of 100 ng/mL when stored in plastic vs glass containers at 4°C in the dark

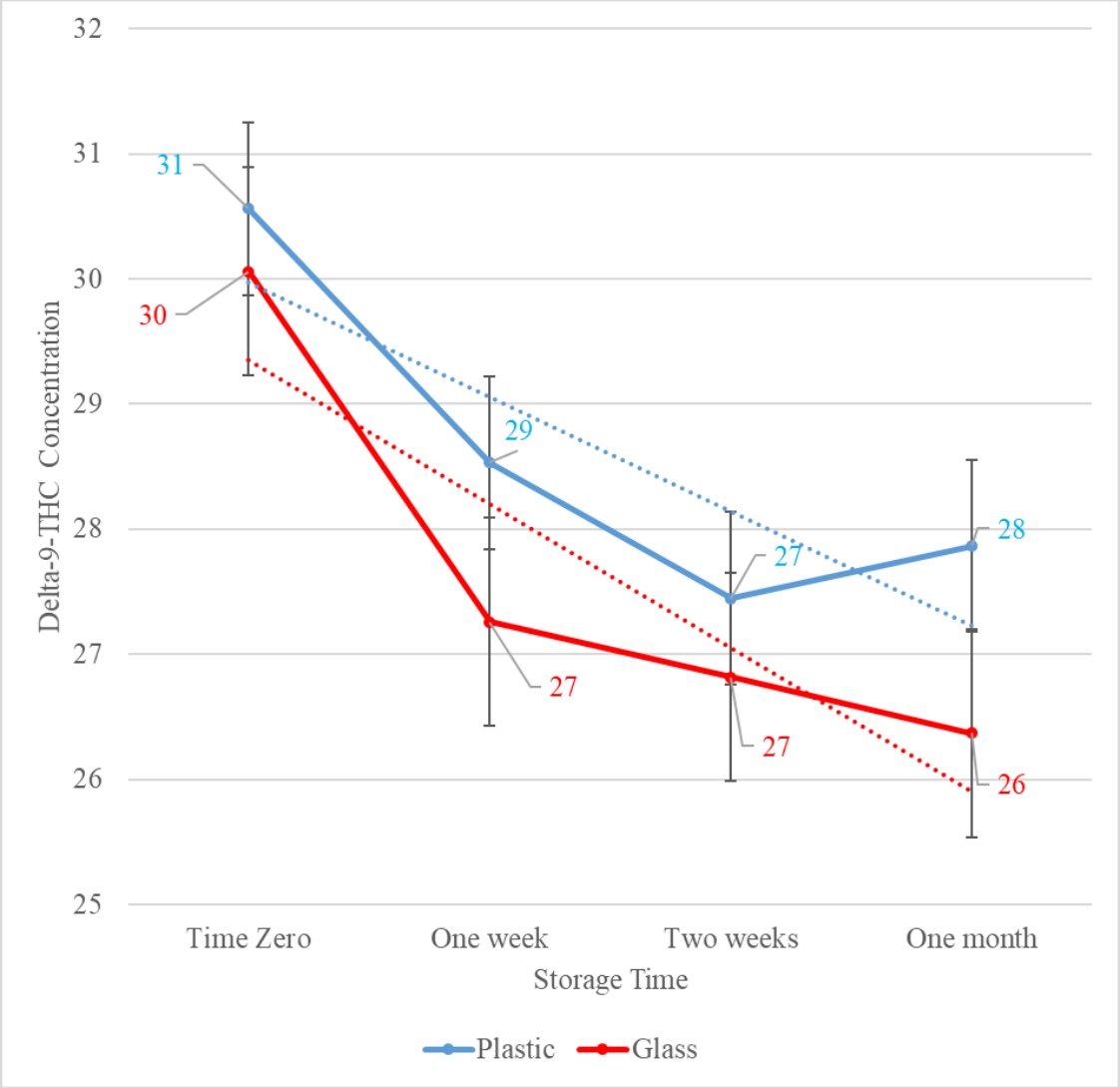

Figure 21. Stability of Δ9-THC in oral fluid spiked at concentrations of 25 ng/mL when stored in plastic vs glass containers at 4°C in the dark

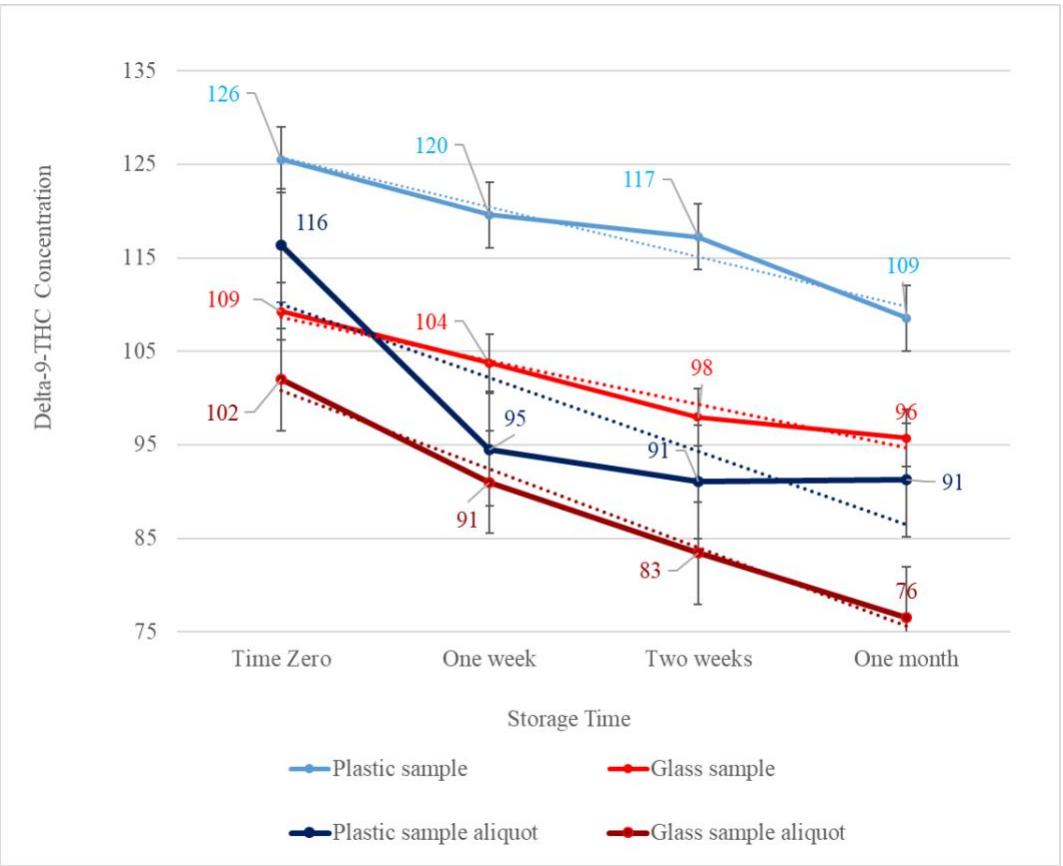

Figure 22. Stability of Δ^9-THC in oral fluid spiked at concentrations of 100 ng/mL in aliquoted vs whole sample in plastic or glass containers when stored at 4°C in the dark

Once an oral fluid sample arrives at the laboratory in the Quantisal® tube, the pad is still in the tube and needs to be plunged, per the manufacturer protocol, to remove all the excess liquid off the pad. The solution is then transferred to either a plastic or glass tube for storage until testing. This study evaluated if storing oral fluid cases positive for Δ^9-THC has any effect on Δ^9-THC stability when stored in the cooler and in the dark for one month. There no substantial difference on Δ^9-THC stability effects when stored in plastic or glass for a month at high and low concentrations. Choi provided that Δ^9-THC losses were less than 10% when stored in glass and greater than 20% when stored in polypropylene plastic tubes after six days of storage.[43] It should be noted that Choi

evaluated the stability of Δ^9-THC in expectorated oral fluid while this study evaluated the stability of oral fluid in the Quantisal® buffer. In comparison, studies have shown that Δ^9-THC has enhanced stability and recovery when stored in glass containers after four weeks compared to plastic containers.[37] The current study demonstrated that it is acceptable to store oral fluid positive for Δ^9-THC in either plastic or glass tubes for up to one month when stored with 3 mL of Quantisal® buffer.

To avoid any Δ^9-THC loss during the freeze/thaw cycle or when removing samples from the respective storage conditions during the analysis at different time points for the 0-90 day, aliquoted sample, stability study, 0.5 mL parts of each sample where separated into separate tubes. The glass versus plastic study evaluated the impact aliquoting the samples may have on Δ^9-THC stability. The lower volumes of sample stored in a tube leads to an increased rate of Δ^9-THC loss. This is consistent with the literature showing that lower volumes of solution have a negative effect on Δ^9-THC stability.[44] With lower volumes of solution and the same concentrations, there is an opportunity for a greater surface area of the drug to be in contact with the sides of the tube. Δ^9-THC is known to be a sticky substance that could be sticking to the tube preventing it from being recovered during the extraction. Even though oral fluid samples stored in Quantisal® buffer in the cooler may have to be removed multiple times for analysis, this method of storage has enhanced stability compared to splitting the sample into 0.5 mL aliquots.

Two Year Stability of Δ⁹-THC Positive Oral Fluid Cases

The stability of Δ⁹-THC in previously analyzed ADFS oral fluid samples that had been stored for two years in the cooler can be seen in Figure 23. The Δ⁹-THC concentration in the cases reanalyzed after two years had concentration decreases ranging from 0.2% to 99.6%. The average and median Δ⁹-THC concentration decrease was 45% after two years of storage. Of the 44 cases reanalyzed, 25 (57%) of those cases had Δ⁹-THC concentration decreases of <50% and 19 (43%) of them decreased by >50%. There were two cases that had Δ⁹-THC concentrations decrease between 91% and 100% and there were three cases that only had a decrease between 0% and 10% demonstrating high variability in stability over long periods.

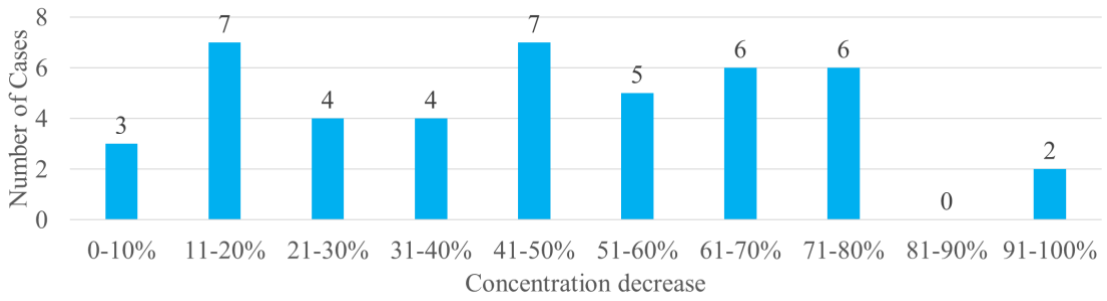

Figure 23. Stability of Δ⁹-THC in previously analyzed oral fluid cases after two years of storage at 4°

Twenty-three percent of samples had Δ⁹-THC concentrations within 20% of the initial analysis concentrations. These 10 oral fluid cases positive for Δ⁹-THC were stable after two years when compared to the initial analysis results. All 10 of the samples that remained stable after two years had initial Δ⁹-THC concentrations less than 100 ng/mL. This indicates that samples of lower concentrations have enhanced stability compared

samples with high concentrations after years of storage. Thirty-four samples had concentration decreases >20%. Every case was still positive for Δ^9-THC after two years except four that were below the LOD for Δ^9-THC upon reanalysis. The statute of limitations for retaining specimens is two years in Alabama, so Δ^9-THC in oral fluid can be detected in most cases if repeated within two years.

Δ^9-THC Stability in the Trunk of a Car (Post-Collection)

Eighteen samples were spiked with Δ^9-THC concentrations of 100 or 25 ng/mL before being stored in a car trunk during the summer and fall for 0-30 days. A control group stored inside the laboratory at room temperature was also tested. Samples were spiked at 100 ng/mL with three being stored at room temperature, three in the trunk during the summer, and three in the trunk during the fall. Samples were spiked at 25 ng/mL with three being stored at room temperature, three in the trunk during the summer, and three in the trunk during the fall. The samples stored at room temperature were subjected to an average temperature of 69°F. The samples stored in the trunk during the summer were subjected to an average temperature of 87°F (range = 68°F-130°F). The samples stored in the trunk during the fall were subjected to an average temperature of 74°F (range = 54°F-107°F). The average concentrations are shown for each storage condition at time zero, one week, and one month. All samples were stored in the dark.

The stability of oral fluid samples spiked at high Δ^9-THC concentrations that were stored in the trunk of a car for up to one month one month are shown in Figure 24. For samples spiked at high Δ^9-THC concentrations and stored at room temperature the average initial concentration for Δ^9-THC was 116 ng/mL. The samples stored at room

temperature in the dark remained stable after one month. The initial average Δ^9-THC concentration for the samples was 108 ng/mL for the samples stored in the trunk during the summer. The Δ^9-THC concentrations for the samples in the trunk during the summer decreased by 48% after one week and 86% after one month. The initial average Δ^9-THC concentration for samples stored in a car trunk during the fall was 116 ng/mL and samples remained stable after one week. Following one month of storage in a trunk during the fall, there was an average concentration decrease of 23%. All samples were unstable in the trunks during the summer and fall after one month of storage.

The stability of Δ^9-THC in oral fluid spiked at low concentrations and stored at room temperature, in a car trunk during the summer, and a car trunk during the fall is shown in Figure 25. The low concentration samples stored at room temperature had an average Δ^9-THC concentration that remained stable after one month of storage. The oral fluid samples stored in the trunk of a car during the fall had average concentration decreases of 8.3% and 27% after one week and one month of storage, respectively. Therefore, it was stable at one week, but unstable at one month. When stored in the trunk during the summer there was an average decrease of 24% and 91% following one week and one month of storage respectively. Samples were unstable at one week.

Recording the date of collection, receipt and first analysis of ADFS oral fluid cases allowed for the number of days between collection and receipt by the lab to be calculated which is shown in Table 5. The average number of days it takes for an oral fluid sample to arrive at the laboratory after collection was 16 days. The median number of days between sample collection and sample receipt was 10 days. The maximum number of days it took for ADFS to receive an oral fluid sample after collection was 247 days. The

mean and median number of days it took for ADFS to analyze an oral fluid sample after it was received was 38 days and 33 days, respectively.

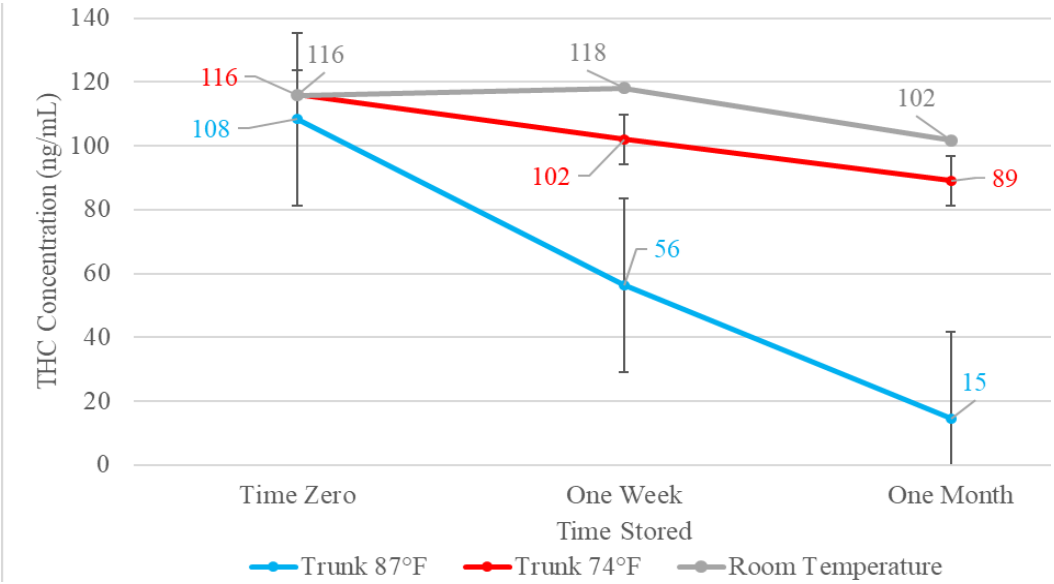

Figure 24. Stability of Δ^9-THC in oral fluid at high concentrations when stored at room temperature or in the trunk of a car during the summer and fall

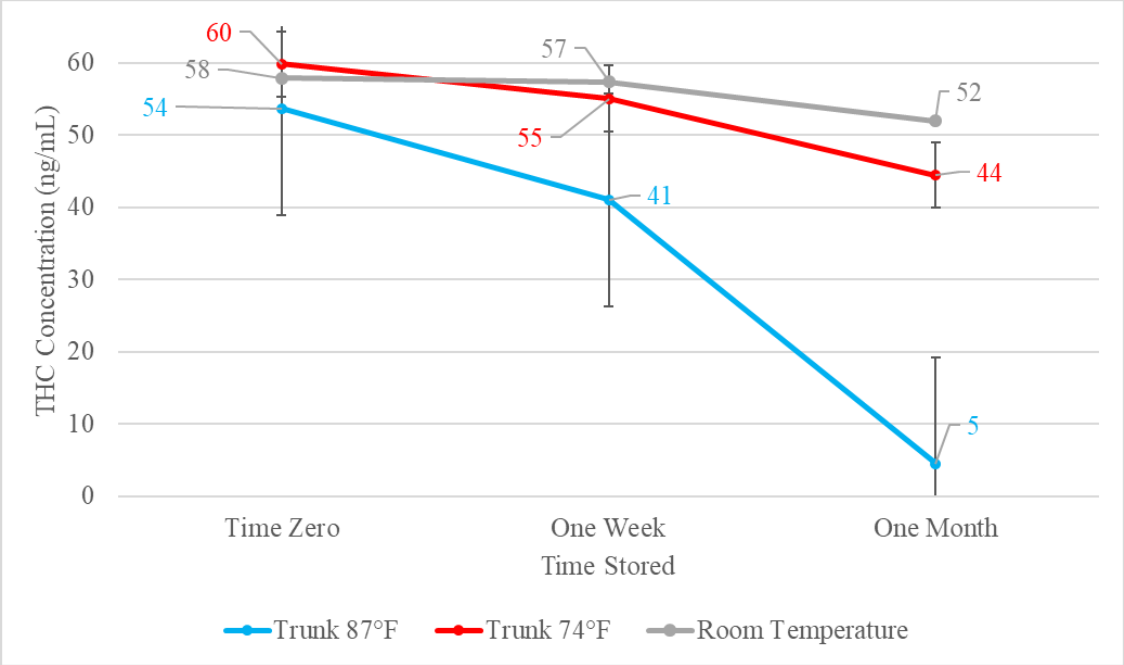

Figure 25. Stability of Δ^9-THC in oral fluid at low concentrations when stored at room temperature or in the trunk of a car during the summer and fall

Table 5. *Number of Days Between Collection of Oral Fluid Sample and First Analysis*

	Time Between Collection and Receipt (Days)	Time Between Receipt and First Analysis (Days)
Average	16	38
Median	10	33
Max	247	168
Min	0	0

It is known that some officers may store evidence (e.g. OF samples) in the trunk of their car with the rest of their equipment for extended time periods. Temperatures in car trunks can become extremely high in certain regions during warm months.

Quantisal® Buffer Stability When Stored in Different Conditions (Pre-Collection)

Six Quantisal® tubes were stored at room temperature (n=2), in the cooler (n=2), or in the trunk of a car (n=2) for one week to evaluate the impact of temperature on the Quantisal® buffer prior to collection. After one week of storage, one Quantisal® device from each condition was used to collect oral fluid with Δ^9-THC concentrations of 100 and one from each condition collected oral fluid with drug concentrations of 25 ng/mL. The reported Δ^9-THC concentration for oral fluid samples collected by Quantisal® devices stored at room temperature, refrigeration, and in the trunk of a car for one week before completing oral fluid collection is shown in Table 6. The average temperature for Quantisal® devices under ambient conditions was 68°F. The refrigerated devices were stored at 39°F. The temperature in the trunk averaged 89°F that ranged from 74°F to 130°F. The oral fluid Δ^9-THC concentration for the Quantisal® stored at refrigeration was not different from the concentration reported for the Quantisal® tubes stored at room temperature or in the trunk before collecting oral fluid spiked at 100 ng/mL of Δ^9-THC. The Δ^9-THC concentration of the collected oral fluid by the Quantisal® device stored in the trunk before collection was not different from than the reported concentration of the oral fluid collected by the devices stored at room temperature or at refrigeration before collecting oral fluid spiked at 25 ng/mL. The Quantisal® buffer is not compromised by

high temperatures. Drugs remain stable in the buffer that was previously exposed to various temperatures prior to oral fluid collection.

Table 6. *Δ^9-THC Concentration Following OF Collection with Quantisal® Devices Stored for One Week in Different Temperatures Prior to Collection*

	High THC Concentration (ng/mL)	Low THC Concentration (ng/mL)
Room Temperature	122	29
Refrigeration	125	29
Trunk of a car	122	30

This study evaluated the question of whether or not storing a Quantisal® tube in the trunk of a car would have an effect on the drug concentration of the oral fluid collected. The packaging of the Quantisal® tube instructs to keep the Quantisal® tube at room temperature, but provides no information on the impact on the tube if stored in warmer or cooler environments. The Quantisal® tubes were stored at room temperature, in the cooler, and in a trunk that ranged from 74°F to 130°F for one week. The Quantisal® tubes then collected expectorant oral fluid spiked at low and high concentrations of Δ^9-THC. The analyzed Δ^9-THC concentrations were all within 2.5% of each other for the different conditions for the samples collected that had high Δ^9-THC concentrations. At

low Δ^9-THC concentrations, the Δ^9-THC concentrations were all within 3.5% of each other for all the samples. Storing Quantisal® tubes in the trunk of a car or in the cooler appears to have no effect on oral fluid Δ^9-THC concentrations collected when stored for a week pre-collection.

Limitations

For the qualitative validation of THC-P and THC-O, one limitation was that ion suppression was not analyzed. Ion suppression should be evaluated to fully validate qualitative methods to make sure no interfereants are altering the responses of the analytes of concern. This research was limited with time that did not allow for ion suppression to be analyzed. More research must be done to evaluate ion suppression since the analytes have similar retention times that may have reduced ionization efficiency. Nonetheless, appropriate LODs were achieved for THC-O and THC-P.

This study was also limited in the fact that only two collection devices were used to evaluate matrix interference. Other oral fluid collection devices need to be evaluated for matrix interference if other devices are to be used by any departments for future oral fluid collection. Expectorant oral fluid should also be evaluated for matrix interference, if that method is used for collection.

Another limitation for this study occurred in the original and repeat stability at 20°C, 4°C, and -20°C studies. Samples stored at -20°C were stored in the dark, but there were times when the light would be flipped on as scientists entered the freezer for other purposes. The light being on for varying intervals throughout the stability study may have had a negative effect on analyte stability. This research showed that light causes faster

rates of Δ^9-THC degradation which could indicate the lights being briefly on in the cooler may have increased the degradation rates of the samples in the freezer for short periods of time throughout the study. Further temperature dependent stability research should ensure the storage conditions are subjected to constant light environments.

One limitation of the trunk stability study is that the oral fluid samples were stored in a vehicle being used for transportation. The vehicle was in use often and was also allowed to remain stationary for several days at a time. The movements of the vehicle could be a factor in Δ^9-THC stability. A vehicle remaining stationary may have enhanced stability compared to a vehicle in use. This study did not document when the vehicle was in use or make note of the temperature in the trunk during these intervals. More research should be done to document the stability of Δ^9-THC when stored in a vehicle that is moving every day or always stationary.

CONCLUSION

Forensic laboratories have a responsibility to be aware of new psychoactive products hitting the market, such as THC-P, and to ensure those substances are not misidentified as controlled compounds. Laboratories also need to stay up to date on the legal status of emerging drugs like THC-O, and develop analytical methods to correctly identify those compounds in future DUID investigations. This study validated a method to detect THC-P and THC-O using an existing cannabinoid oral fluid extraction method.

A second issue addressed in this research was Δ^9-THC stability under different storage conditions. It is important to understand Δ^9-THC stability in order to ensure samples are stored in such a way that the results reflect the true nature of a sample. This study shows that Δ^9-THC is most stable in oral fluid when stored in the dark. Storing the oral fluid samples in plastic versus glass containers had no impact on Δ^9-THC stability. Oral fluid samples suspected of being positive for cannabinoids should be stored at 4°C, in the dark, and as a whole (non-aliquoted) sample with the 3 mL of Quantisal® buffer. Oral fluid samples should not be split into several aliquots due to increased surface area available for Δ^9-THC binding. The lower the sample volume in a tube, the higher the THC concentration decrease expected to occur during storage. Samples with higher concentrations of Δ^9-THC should be expected to have less stability compared to samples of low concentrations. Oral fluid samples should not be subjected to light exposure and warm temperatures if possible. The Quantisal® tubes can be stored in the trunk of a car

pre-collection for up to a week without an effect on the Δ^9-THC concentration of the oral fluid sample, unless at temperatures exceeding 130°F.

More research needs to be conducted on the stability of novel cannabinoids in different conditions to fully understand if they have similar stabilities to Δ^9-THC in oral fluid. This may also further explain why the concentrations of THC-O increased more than 20% during the course of this stability study. Further research should also be done to see how stability of Δ^9-THC is affected when stored in a trunk during the winter and to see if there is any impact on using Quantisal® tubes stored in a trunk longer than a week for oral fluid collection. Finally, more cannabinoids are being produced and forensic laboratories should consider adding emerging cannabinoids like Tetrahydrocannabivarin (THC-V) and Hexahydrocannabinol (HHC) to current THC methods. As HHC was included in the DEA report substances classified as Schedule I, it should be next on the list of targets to validate in cannabinoid analysis methods.

In summary, oral fluid proves to be a viable specimen for DUI applications. However, care should be taken when cannabinoids are suspected. There should be timely collection, submission, and testing of these samples. It is recommended to store oral fluid samples at refrigeration and in the dark prior to testing.

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APPENDIX A

Structures of Δ^9-THC, Δ^9-THC-P and Δ^9-THC-O

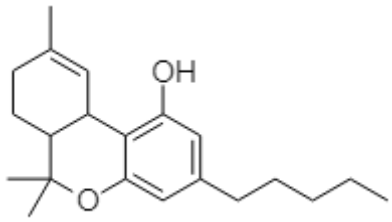

Figure A1. Δ^9-THC

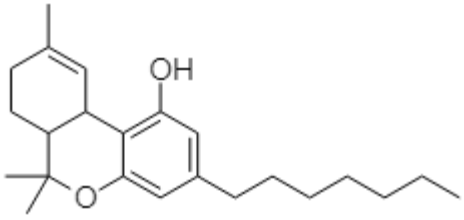

Figure A2. Δ^9-THC-P

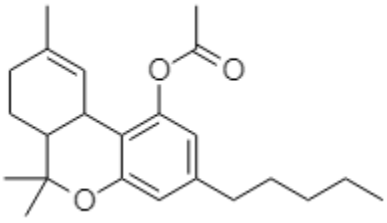

Figure A3. Δ^9-THC-O

APPENDIX B

List of Commonly Encountered Analytes Evaluated for Interference

Table A1. *List of Commonly Encountered Targets at 100 ng/mL*

Analyte	Cannabinoids Evaluated Against
6-MAM	Δ^9-THC
7-Aminoflunitrazepam	Δ^8-THC
Acetyl Fentanyl	9R-Δ^{10}-THC
Acryl Fentanyl	9S-Δ^{10}-THC
Alprazolam	Δ^9-THC-P
Amphetamine	Δ^8-THC-P
AP 238	Δ^9-THC-O
Benzoylecgonine	Δ^8-THC-O
Bromazepam	THC-OH
Bromazolam	THC-COOH
Brorphine	CBD
Butonitazene	CBG
Butyryl Fentanyl	CBN
Carfentanil	
Chlordiazepoxide	
Clobazam	
Clonazepam	
Cocaethylene	
Cocaine	
Codeine	
Cyclopropyl Fentanyl	
Delorazepam	
Deschloroetizolam	
Diazepam	
Diclazepam	
Estazolam	
Etizolam	
Etodesnitazene	
Etonitazepyne	
Fentanyl	
Flualprazolam	
Flubromazepam	
Flubromazolam	
Flunitrazepam	
Fluorofentanyl	
Fluoroisobutyryl Fentanyl	
Flutoprazepam	
Furanyl Fentanyl	

Hydrocodone
Hydromorphone
Isotonitazene
Lorazepam
Meclonazepam
Meperidine
Methadone
Methamphetamine
Methoxyacetyl Fentanyl
Midazolam
Mitragynine
Morphine
Nordiazepam
Oxazepam
Oxycodone
Oxymorphone
Phenazepam
Protonitazene
Pyrazolam
Tapentadol
Temazepam
Tianeptine
Triazolam
U-47700
Valeryl Fentanyl
Zaleplon
Zolpidem

APPENDIX C

Initial Δ^9-THC Concentration for Each Sample in Non-Aliquoted Sample 30 Day Stability Study

Table A2. *Initial Δ^9-THC Concentration for Stability Study*

Sample ID	Spiked Concentration (ng/mL)	Initial Concentration (ng/mL)
20°C A	200	114
20°C B	200	116
20°C C	200	80
20°C D	50	18
20°C E	50	27
20°C F	50	34
4°C A	200	87
4°C B	200	101
4°C C	200	95
4°C D	50	27
4°C E	50	21
4°C F	50	19
-20°C A	200	78
-20°C B	200	97
-20°C C	200	98
-20°C D	50	21
-20°C E	50	22
-20°C F	50	22

APPENDIX D

Number of Samples at Each Experimental Condition

Table A3. *Number of Samples at Each Experimental Condition in All Studies*

Study	Targets	Spiked Concentration (ng/mL)	Temp	Non-Aliquoted or Aliquoted Sample	Stored in Light or Dark	Storage container	Number of Samples
30-Day Non-Aliquoted Stability	Δ^9-THC	200	20°C	non-aliquoted	dark	plastic	3
30-Day Non-Aliquoted Stability	Δ^9-THC	50	20°C	non-aliquoted	dark	plastic	3
30-Day Non-Aliquoted Stability	Δ^9-THC	200	4°C	non-aliquoted	dark	plastic	3
30-Day Non-Aliquoted Stability	Δ^9-THC	50	4°C	non-aliquoted	dark	plastic	3
30-Day Non-Aliquoted Stability	Δ^9-THC	200	-20°C	non-aliquoted	dark	plastic	3
30-Day Non-Aliquoted Stability	Δ^9-THC	50	-20°C	non-aliquoted	dark	plastic	3
90-Day Aliquoted Stability	Δ^9-THC and Δ^8-THC	200	20°C	aliquoted	dark	plastic	3
90-Day Aliquoted Stability	Δ^9-THC and Δ^8-THC	50	20°C	aliquoted	dark	plastic	3

90-Day Aliquoted Stability	Δ^9-THC-P, Δ^8-THC-P, and THC-O	200	20°C	aliquoted	dark	plastic	3
90-Day Aliquoted Stability	Δ^9-THC-P, Δ^8-THC-P, and THC-O	50	20°C	aliquoted	dark	plastic	3
90-Day Aliquoted Stability	Δ^9-THC and Δ^8-THC	200	4°C	aliquoted	partial light	plastic	3
90-Day Aliquoted Stability	Δ^9-THC and Δ^8-THC	50	4°C	aliquoted	partial light	plastic	3
90-Day Aliquoted Stability	Δ^9-THC-P, Δ^8-THC-P, and THC-O	200	4°C	aliquoted	partial light	plastic	3
90-Day Aliquoted Stability	Δ^9-THC-P, Δ^8-THC-P, and THC-O	50	4°C	aliquoted	partial light	plastic	3
90-Day Aliquoted Stability	Δ^9-THC and Δ^8-THC	200	-20°C	aliquoted	dark	plastic	3
90-Day Aliquoted Stability	Δ^9-THC and Δ^8-THC	50	-20°C	aliquoted	dark	plastic	3
90-Day Aliquoted Stability	Δ^9-THC-P, Δ^8-THC-P, and THC-O	200	-20°C	aliquoted	dark	plastic	3

90-Day Aliquoted Stability	Δ^9-THC-P, Δ^8-THC-P, and THC-O	50	-20°C	aliquoted	dark	plastic	3
Light vs Dark	Δ^9-THC	100	4°C	non-aliquoted	dark	plastic	3
Light vs Dark	Δ^9-THC	25	4°C	non-aliquoted	dark	plastic	3
Light vs Dark	Δ^9-THC	100	4°C	non-aliquoted	light	plastic	3
Light vs Dark	Δ^9-THC	25	4°C	non-aliquoted	light	plastic	3
Plastic vs Glass	Δ^9-THC	100	4°C	non-aliquoted	dark	plastic	3
Plastic vs Glass	Δ^9-THC	25	4°C	non-aliquoted	dark	plastic	3
Plastic vs Glass	Δ^9-THC	100	4°C	non-aliquoted	dark	glass	3
Plastic vs Glass	Δ^9-THC	25	4°C	non-aliquoted	dark	glass	3
Non-Aliquoted vs Aliquoted	Δ^9-THC	100	4°C	aliquoted	dark	plastic	3
Non-Aliquoted vs Aliquoted	Δ^9-THC	100	4°C	aliquoted	dark	glass	3
Trunk Stability	Δ^9-THC	100	20°C	non-aliquoted	dark	plastic	3
Trunk Stability	Δ^9-THC	50	20°C	non-aliquoted	dark	plastic	3
Trunk Stability	Δ^9-THC	100	20°C-54°C	non-aliquoted	dark	plastic	3
Trunk Stability	Δ^9-THC	50	20°C-54°C	non-aliquoted	dark	plastic	3
Trunk Stability	Δ^9-THC	100	12°C-42°C	non-aliquoted	dark	plastic	3

Trunk Stability	Δ^9-THC	50	12°C-42°C	non-aliquoted	dark	plastic	3
Quantisal Buffer Stability	Δ^9-THC	100	20°C	non-aliquoted	dark	plastic	1
Quantisal Buffer Stability	Δ^9-THC	25	20°C	non-aliquoted	dark	plastic	1
Quantisal Buffer Stability	Δ^9-THC	100	4°C	non-aliquoted	dark	plastic	1
Quantisal Buffer Stability	Δ^9-THC	25	4°C	non-aliquoted	dark	plastic	1
Quantisal Buffer Stability	Δ^9-THC	100	23°C-54°C	non-aliquoted	dark	plastic	1
Quantisal Buffer Stability	Δ^9-THC	25	23°C-54°C	non-aliquoted	dark	plastic	1