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Assessing the Detectability of Cannabinoid Analogs (Delta-8 THC, Delta-10 THC and CBD) and their Major Metabolites in Six Commercial Cannabinoid Urine Screening Kits

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"Assessing the Detectability of Cannabinoid Analogs (Delta-8 THC, Delta-10 THC and CBD) and their Major Metabolites in Six Commercial Cannabinoid Urine Screening Kits"

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ABSTRACT

Background:

There has been an exponential surge in the presence and use of cannabinoids since the federal legalization of hemp (Agricultural Improvement Act of 2018), which involves the legalization of medical use, adult recreational use, and decriminalization of cannabis in several states. This growth is not only attributed to Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), the most abundant phytocannabinoid components of cannabis and hemp, respectively, but with many other emerging THC analogs. Structurally, these analogs are similar to Δ9-THC, yet very little information is available about their potency and even less information is available regarding their detectability using commercially available cannabinoid screening kits. Due to their structural similarity, current Δ9-THC immunoassay screening methods may be able to detect these emerging cannabinoid analogs.

Objectives:

To evaluate the ability of six commercially available homogeneous urine cannabinoid screening kits to detect Δ8-THC, CBD and their major metabolites, and Δ10-THC chiral analogs at 50 ng/mL and 20 or 25 ng/mL cutoff concentrations.

Methods:

Six urine immunoassay kits (Abbott Cannabinoids – Abbott Diagnostics, LZI Cannabinoids (cTHC) Enzyme Immunoassay – Lin-Zhi International, DRI® Cannabinoid Assay and CEDIA™ THC – Thermo Fisher Scientific, ONLINE DAT Cannabinoid II – Roche Diagnostics, and Syva EMIT®II Plus – Siemens Healthineers) were evaluated at two different cutoff concentrations: 50 ng/mL and 20 or 25 ng/mL. The analysis was performed on an Abbott Architect Plus c4000 (Abbott Diagnostics). Δ8-THC, CBD, olivetol and their major metabolites, and Δ10-THC chiral analogs were evaluated. The limit of detection was evaluated by preparing each analyte at 20, 50, 100, and 1000 ng/mL in urine. Samples were analyzed at both cutoff concentrations to determine if the analyte could be detected at one or both cutoff(s). Analytes not detected at 1000 ng/mL for a cutoff were considered not detectable. If detected, the appropriate concentration was used as the decision point to determine the precision at the immunoassay's cutoff. Precision was assessed using three QC pools of the analyte prepared at -50%(QCN), the decision point, and +100%(QCP), which were analyzed in five different runs (n=3) along with the respective immunoassay's control materials. The total mean (n=15), standard deviation (SD), and percent coefficient of variation (%CV) were calculated for each QC concentration. A decision point was considered valid if the %CV for the QC was ≤20% for each concentration, and the total mean of the QCN and QCP ±2SD did not overlap the mean of the decision point.

Results:

The minimum detectable concentration for Δ8-THC was 200 ng/mL using the 50 ng/mL cutoff by Abbott, DRI, LZI, and SYVA. The minimum detectable concentration for Δ8-THC was 100 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), DRI(20), LZI(25), and SYVA(20). Δ8-THC was not detected at either cutoff by CEDIA or ROCHE. The minimum detectable concentration for 11-OH-Δ8-THC and 11-COOH-Δ8-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, ROCHE, and SYVA. The minimum detectable concentration for 11-OH-Δ8-THC and 11-COOH-Δ8-THC was 50 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), ROCHE(20), and SYVA(20).

The minimum detectable concentration for 6-OH-CBD was 1000 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), DRI(20), LZI(25), and ROCH(20). The minimum detectable concentration for 7-OH-CBD was 1000 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), DRI(20), and LZI(25). 6-OH-CBD and 7-OH-CBD were not detected by any of the six immunoassays at the 50 ng/mL cutoff. CBD, 7-COOH-CBD, Abn-CBD, and CBDA-A were not detected by any of the six immunoassays at either the 50 ng/mL or 20 or 25 ng/mL cutoffs.

The four Δ10-THC analogs were detected at 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, and SYVA. The four Δ10-THC analogs were detected at 50 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), and SYVA(20). None of the analogs were detected by ROCHE(20) and ROCHE(50).

Olivetol was only detected at 1000 ng/mL by the ROCHE screening kit at both the 50 ng/mL and 20 or 25 ng/mL cutoffs. Olivetolic acid was not detected by any of the six immunoassays at either the 50 ng/mL or 20 or 25 ng/mL cutoffs.

Conclusions:

The six commercially available homogeneous urine cannabinoid screening kits were able to detect Δ8-THC, 11-OH-Δ8-THC, 11-COOH-Δ8-THC, 6-OH-CBD, 7-OH-CBD, all Δ10-THC chiral analogs, and olivetol with varying selectivity depending on the screening kit. The six commercially available homogeneous urine cannabinoid screening kits were not able to detect CBD, 7-COOH-CBD, Abn-CBD, CBDA-A, and olivetolic acid.

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1 INTRODUCTION

1.1 Background

There has been an exponential surge in the presence and use of cannabinoids since the federal legalization of hemp (Agricultural Improvement Act of 2018), which involves the legalization of medical use, adult recreational use, and decriminalization of cannabis in several states. This growth is not only attributed to Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), the most abundant phytocannabinoid components of cannabis and hemp, respectively, but also other emerging THC analogs. Recently, Δ8-tetrahydrocannabinol (Δ8-THC) and Δ10-tetrahydrocannabinol (Δ10-THC) have become readily available commercially in various products including e-cigarettes, edibles, and powders, etc. Moreover, these products are easily accessible and can be purchased online readily or found in local retail shops such as gas stations, vape shops, and other businesses that sell drug paraphernalia.

Federally, the US Drug Enforcement Administration (DEA) is responsible for the regulation of cannabinoids and cannabinoid-containing-products, and marijuana has been classified as a Schedule I drug/substance. However, the US Food and Drug Administration (FDA) has approved prescription formulations of CBD (Epidiolex®) and THC (Marinol®) that are legal for the treatment of certain medical disorders. Currently, both Δ8-THC and Δ10-THC are considered legal and have the potential for abuse. These analogs can bind to and activate the CB1 cannabinoid receptor, which is responsible for producing psychoactive effects like elation and relaxation. Limited studies have reported that Δ8-THC has similar potency when compared to Δ9-THC, however very little information is available regarding the potency of Δ10-THC. At this given time, it is not possible to distinguish the source of Δ8-THC since it may be extracted from hemp, which is legal, or synthesized from hemp-derived CBD, which is illicit. This not only opens doors for

consumers to obtain Δ8-THC readily, licitly or illicitly, but it also creates uncertainty in regard to its legal status. Due to Δ8-THC's regulatory ambiguity and its psychoactive effects, it has become a popular product online and in vape shops.

Δ8-THC, CBD, and Δ10-THC are constitutional isomers of Δ9-THC ($C_{21}H_{30}O_2$, Molecular Weight (MW) 314), as are their carboxylic metabolites ($C_{21}H_{28}O_4$, MW 344). Furthermore, the structural difference between Δ8-THC, Δ9-THC, and Δ10-THC is limited to the location of a single double-bond. For that reason, this may make their detection amenable to current immunoassays used for the detection of Δ9-THC-Carboxylic Acid (Δ9-THC-COOH) in urine. With Δ8-THC consumption steadily rising, this poses a forensic issue as Δ8-THC may potentially cross-react with current commercially available immunoassays, which are commonly employed in forensic, clinical, and pain management laboratories. Therefore, elucidating the nature of this interaction would be immensely valuable to laboratories that commonly employ urine cannabinoid testing in order to gain insights into the potential for false-positive THC screens. This information is hugely important as it can result in costly increases in the number of confirmatory testing required. Due to Δ8-THC's commercial availability, Δ8-THC consumption is steadily rising, which is an indication that the rise in testing costs may be substantial.

A publication recently indicated that seized materials from DUI traffic stops have occasionally (3.7%) tested positive for an interfering chromatographic peak which was later identified as Δ8-THC.[1] Forensically, this is significant since Δ8-THC (not currently scheduled) may be falsely identified as Δ9-THC (scheduled), which can subsequently lead to false prejudicial conclusions, or incorrect assumptions that have legal ramification for an individual. The majority of forensic urine drug testing occurs in the workplace, human performance, court ordered (i.e.,

[1] Chan-Hosokawa et al., "Emergence of Delta-8 Tetrahydrocannabinol in DUID Investigation Casework."

probation/parole, incarceration, and child custody), US Military, US Department of Transportation, etc., which follows the adopted federal regulations as the basis for testing protocols. Per the Federal Register, "the purpose of the workplace drug testing program is to deter and detect use of illegal drugs".[2] With that being said, a false positive result can result in someone potentially losing their job, going back to jail, or losing a child. Currently, the Medical Review Officer Manual[3] lists delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA or THC-COOH) as the only cannabinoid involved in testing and to report a positive result, "a specimen must test positive at or above the 50 ng/mL cutoff for the initial test and have a concentration of the delta-9 THCA that is equal to or greater than the 15 ng/mL confirmatory cutoff level."[4] Given the structural similarities of emerging THC analogs, it is likely that these analogs and/or their metabolites may cause false positive screenings for current commercially available homogeneous immunoassays utilized to detect Δ9-THC-COOH. In December of 1998, the US Health and Human Services increased the screening cutoff for opiates from 300 ng/mL to 2000 ng/mL "to eliminate most specimens that test positive due to poppy seed ingestion or due to the use of legitimate morphine or codeine medication."[2,3,5] It is possible that Δ9-THC-COOH screening cutoffs may increase as well to account for consumption of other analogs if it is found that there is potential for cross-reactivity.

Drug testing usually begins with a screening method, which is utilized to quickly and inexpensively exclude negative specimens from presumptive positives that require further time consuming and costly confirmatory testing. For cannabinoid/marijuana abuse testing, Δ9-THC-COOH, the main metabolite of Δ9-THC, has been the standard compound utilized for detection.

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[2] Mandatory Guidelines for Federal Workplace Drug Testing Programs, SAMSHA, 1994
[3] Medical Review Officer Manual for Federal Agency Workplace Drug Testing Programs, Department of Health and Human Services Substance Abuse and Mental Health Services Administration Center for Substance Abuse Prevention
[4] Mandatory Guidelines for Federal Workplace Drug Testing Programs, SAMSHA, 1997
[5] Substance Abuse and Mental Health Services Administration. Clinical Drug Testing in Primary Care. TAP.

In this case, urine is the matrix of choice as it can be fairly easily obtained in a large quantity, and it allows for a long window of detection of drug use.[6] The federal screening cutoff for Δ9-THC-COOH is 50 ng/mL, however in certain cases, a lower cutoff (20 or 25 ng/mL) is used to further extend the detection window.[4,6] Currently, there is little to no published information on the cross-reactivity of THC analogs[7] and their metabolites[8,9] with commercial urine drug testing kits. Information that is available for THC analog testing utilizes liquid chromatography coupled to mass spectrometry (LCMS), however this method is not cost-effective in terms of screening. Most drug testing laboratories utilize liquid homogeneous immunoassay cannabinoid (HEIC) methods for screening because they require no pretreatment of the urine. Moreover, these methods are adaptable to automated immunoassay analyzers, which cuts down the screening time as hundreds to thousands of tests can be performed in an hour and they require less technical skills than LCMS testing.

Currently, there is very limited antidotal information available from various forensic laboratories, including the FIRM Specialty Testing Laboratory at VCU Health, that suspect that some commercially available HEICs have the potential to cross-react with emerging THC analogs. Given the limited information available, the aim of this study is to evaluate the ability to detect THC analogs (Δ8-THC, CBD, and Δ10-THC) and their biological urine metabolites using commercially available HEICs. The results of this project will increase the knowledge of the forensic science community by increasing the basic understanding and capabilities of current

[6] Cary PL, Drug Court Practitioner, "Fact Sheet: The marijuana detection window: determining the length of time cannabinoids will remain detectable in urine following smoking: A critical review of relevant research and cannabinoid detection guidance for drug courts."
[7] Kroner GM et al., "Cannabinol (CBN) cross-reacts with two urine immunoassay designed to detect tetrhydrocannabinol (THC) metabolite."
[8] Grafinger KE et al., "Determination of the cross-reactivity of the biological metabolite (−)-trans-□9-tetrahydrocannabinol-carboxylic acid-glucuronide (THC-COOH-Gluc) for cannabinoid immunoassays."
[9] Watanabe K et al., "Cross-reactivity of various tetrahydrocannabinol metabolites with a monoclonal antibody against tetrahydrocannabinolic acid."

homogeneous cannabinoid immunoassay screening assays to detect the increasing number of THC analogs being encountered. This will assist the forensic science and regulatory community understand the ability of routine urine cannabinoid drug testing to detect these analogs and what potential interference they may have on cannabinoid testing results.

1.2 Metabolism Mechanisms for THC Analogs

The metabolic pathways for each parent compound (Δ8-THC, CBD, and Δ10-THC) explored in this study will be discussed in this section. This information is relevant as it outlines the parent compounds and their corresponding biological metabolites that will be tested in this study.

1.2.1 Δ8-THC

Δ8-THC has two major metabolic products: 11-OH-Δ8-THC and 11-COOH-Δ8-THC. The methyl group in the 11th position on the parent compound, Δ8-THC, gets hydroxylated to form the first metabolic product 11-OH-Δ8-THC. 11-OH-Δ8-THC gets further metabolized to form 11-COOH-Δ8-THC, which is Δ8-THC's main metabolite.[10] The metabolic scheme for Δ8 THC can be found below, in Figure 1.

[10] Watanabe K et al., "Metabolic disposition of delta 8-tetrahydrocannabinol and its active metabolites, 11-hydroxy-delta 8-tetrahydrocannabinol and 11-oxo-delta 8-tetrahydrocannabinol, in mice."

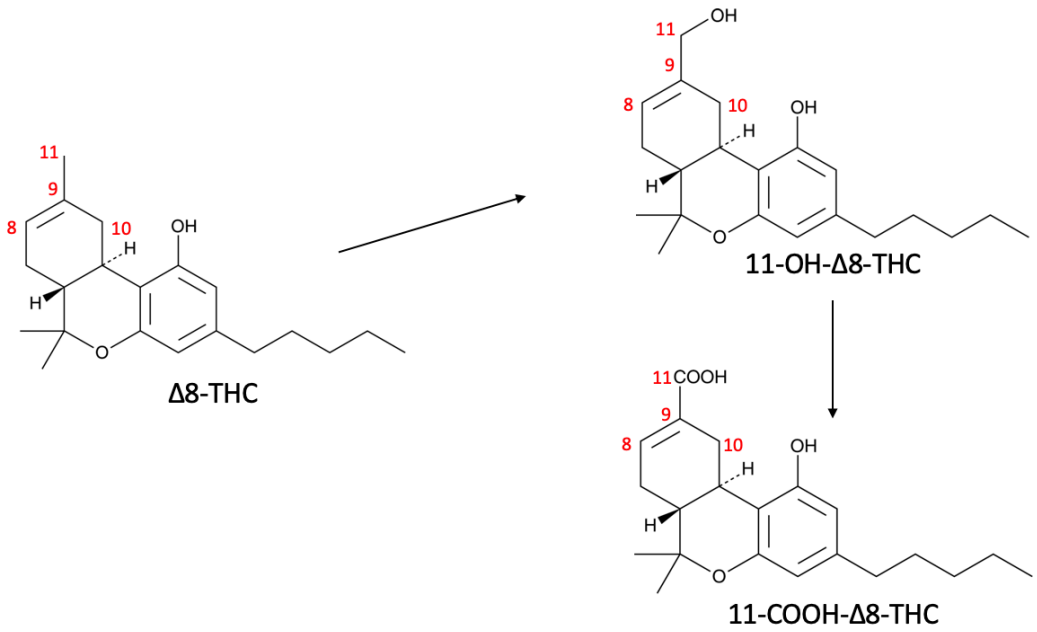

Figure 1. Δ8-THC Metabolism

1.2.2 CBD

CBD has three major metabolic products: 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD. In the 6th position on the parent compound, CBD, a hydroxyl group is added. No further metabolization occurs in this particular position as it does not have a free carbon for a carboxylic acid group to attach to. The methyl group in the 7th position on the parent compound, CBD, gets hydroxylated to form 7-OH-CBD, which undergoes further metabolization to form 7-COOH-CBD.[11] The metabolic scheme for CBD can be found below, in Figure 2.

[11] Beers, Fu, and Jackson, "Cytochrome P450–Catalyzed Metabolism of Cannabidiol to the Active Metabolite 7-Hydroxy-Cannabidiol."

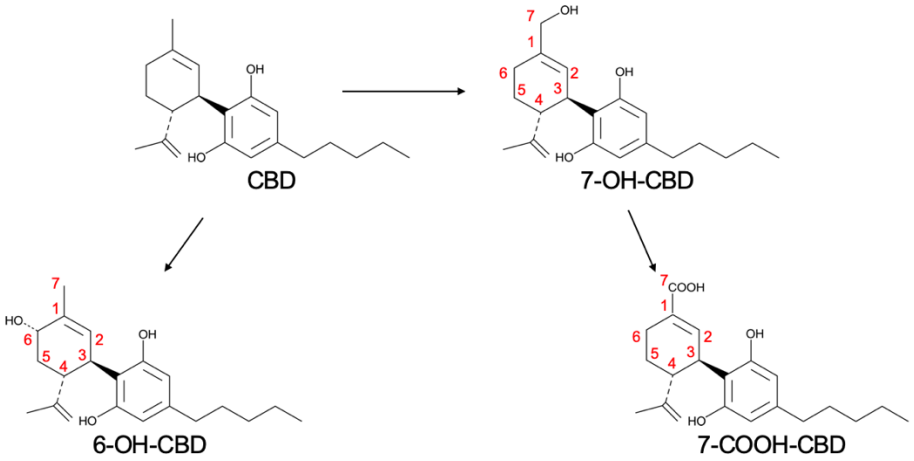

Figure 2. CBD Metabolism

CBD has another carboxylic acid compound, which is known as CBDA-A.[12] CBDA-A is naturally occurring in the plant and it is an inactive compound. The structure of CBDA-A can be found in Figure 3, below.

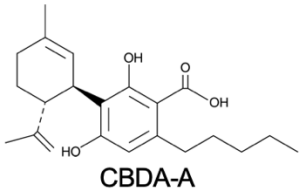

Figure 3. Structure of CBDA-A

CBD also has a synthetic regioisomer known as abnormal-CBD (Abn-CBD).[13] A regioisomer is a class of constitutional isomers which have the same functional groups but attached at different positions. The positional differences between CBD and Abn-CBD are highlighted in the scheme below, in Figure 4.

[12] Gagne et al., "Identification of Olivetolic Acid Cyclase from Cannabis Sativa Reveals a Unique Catalytic Route to Plant Polyketides

[13] Szczesniak et al., "Nonpsychotropic Cannabinoids, Abnormal Cannabidiol and Canabigerol-Dimethyl Heptyl, Act at Novel Cannabinoid Receptors to Reduce Intraocular Pressure.

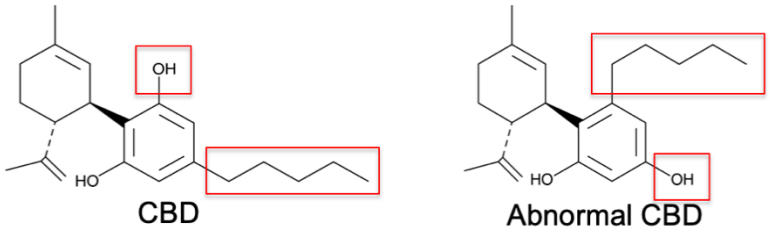

Figure 4. CBD vs. Abn-CBD

1.2.3 Δ10-THC

Δ10-THC is a chiral compound and it exists as four different compounds: 9(R)-$\Delta^{6a,10a}$-THC, 9(S)-$\Delta^{6a,10a}$-THC, (6aR,9R)-Δ^{10}-THC, and (6aR,9S)-Δ^{10}-THC.[14] Their structures are shown, below, in Figure 5. Currently, the metabolic pathway for the four chiral Δ10-THC analogs is not characterized, therefore the metabolites are not commercially available. For this reason, Δ10-THC's metabolites will not be analyzed in this study.

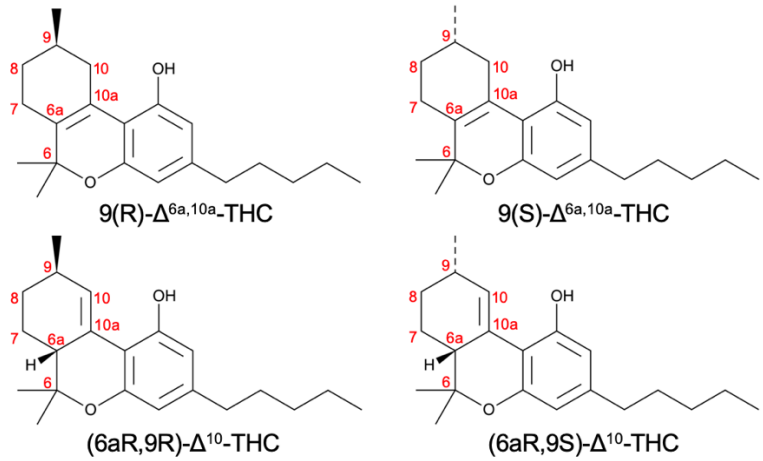

Figure 5. Structures of the four chiral Δ10-THC analogs.

1.2.4 Olivetol

Olivetol[12] is a precursor in various syntheses of THC, which is why it was tested as part of the scope for this project. It has one major metabolite, olivetolic acid. Olivetolic acid is formed

[14] Schafroth et al., "Δ9-Cis-Tetrahydrocannabinol: Natural Occurrence, Chirality, and Pharmacology."

when the parent, olivetol, undergoes carboxylation. The metabolic scheme for olivetol can be found below, in Figure 6.

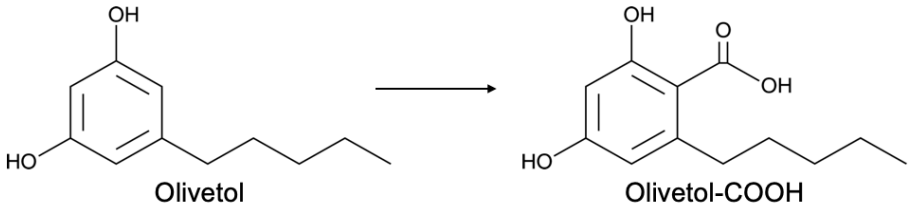

Figure 6. Olivetol Metabolism

Figure 7, summarizes the proposed cannabinoid biosynthetic pathway of Δ9-THC and CBD from olivetolic acid.[12] Olivetolic acid is first converted to cannabigerolic acid (CBG). The pathway then divides leading to the major cannabinoids Δ9-THCA and CBDA. Δ9-THC and CBD are then yielded through a simple decarboxylation.

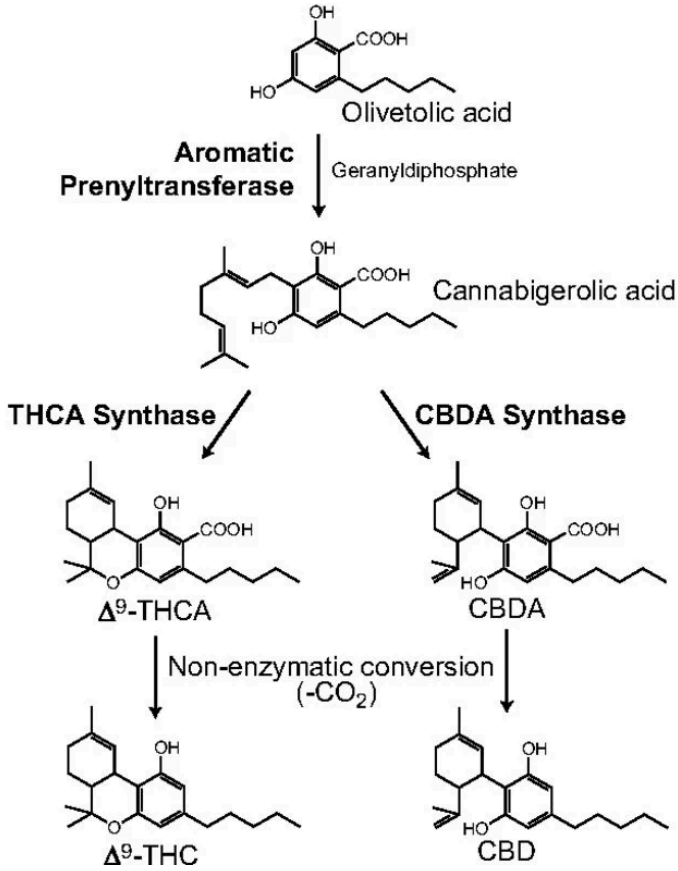

Figure 7. Proposed Synthesis of Δ9-THC and CBD from Olivetolic Acid.

2 RESEARCH MATERIALS & METHODS

2.1 Set-Up and Verification of HEICs

Six commercially available liquid enzymatic immunoassay methods for the detection of cannabinoids (HEIC) in urine at the federal (50 ng/mL) and lower (20 or 25ng/mL) screening cutoff concentrations were setup and verified following AAFS Standards Board Standard 036, Standard Practices for Method Validation in Forensic Toxicology.[15] All of the analyses were performed on an Abbott Architect Plus c4000 (Abbott Diagnostics).

The following six urine immunoassay kits were utilized: Abbott Cannabinoids– Abbott Diagnostics, LZI Cannabinoids (cTHC) Enzyme Immunoassay– Lin-Zhi International, DRI® Cannabinoid Assay and CEDIA™ THC – Thermo Fisher Scientific, ONLINE DAT Cannabinoid II – Roche Diagnostics, and Syva EMIT®IIPlus – Siemens Healthineers. Each kit was verified for their ability to detect cannabinoids utilizing the manufacturer's parameters and procedures.

Figure 5,[16] below, summarizes the five commonly employed homogeneous competitive assays currently available on the market for urine drug screening, fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), luminescent oxygen channeling immunoassay (LOCI), kinetic interaction of microparticle in solution (KIMS), and cloned enzyme donor immunoassay (CEDIA). Abbott Cannabinoids (ABBOTT), LZI Cannabinoids (cTHC) Enzyme Immunoassay (LZI), DRI® Cannabinoid Assay (DRI), and Syva EMIT®IIPlus (SYVA) are EMIT based HEICs. ONLINE DAT Cannabinoid II (ROCHE) is a KIMS based HEIC. CEDIA™ THC (CEDIA) is a CEDIA based HEIC. Since these three assay techniques are employed by the kits utilized in this study, they will be further discussed.

[15] Standard Practices for Method Validation in Forensic Toxicology, ASB Standard 036, 1st Ed. 2018
[16] Sanavio and Krol, "On the Slow Diffusion of Point-of-Care Systems in Therapeutic Drug Monitoring."

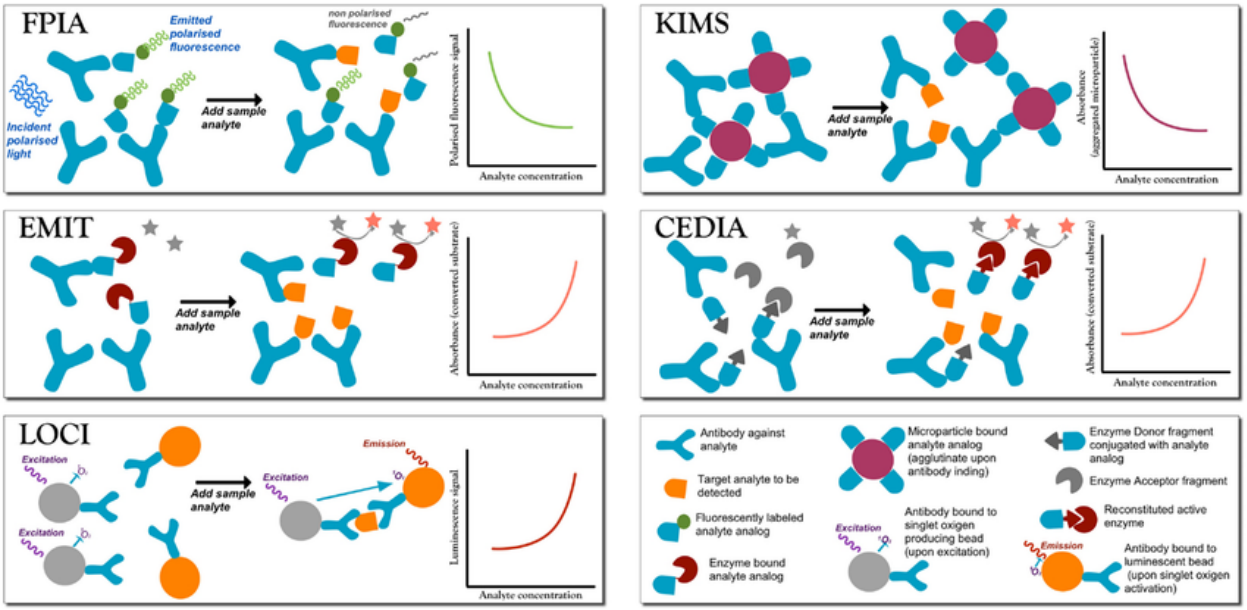

Figure 8. Commonly Employed Homogeneous Competitive Assays.

2.1.1 EMIT

In an EMIT assay, drug analog molecules labeled with an enzyme are added to the test solution to compete against the free drug for binding. When the antibody is bound to the enzyme-drug-conjugate this inhibits the enzymes activity, which prevents NAD to bind to the enzyme thus hindering it from producing NADH. When the antibody is bound to the free drug in the sample, the enzyme-drug-conjugate is uninhibited and can bind to NAD to produce NADH. This increased conversion of NAD to NADH can be measured at the 340 nm wavelength and the signal intensity is directly proportional to the free drug concentration in the solution.

2.1.2 KIMS

In a KIMS assay, in the absence of free drug, the antibody binds to drug-microparticle-conjugates to form aggregates. The aggregates absorb light in the visible range and this absorbance is monitored. In presence of the free drug, the aggregate formation is disrupted as the antibody competitively binds to the free drug and not the drug-microparticle-conjugate, subsequently leading to a reduction in absorbance, which can be measured at the 572 nm wavelength. The signal

generated is inversely proportional to the free drug concentration, which is opposite when compared to EMIT.

2.1.3 CEDIA

In a CEDIA assay, an enzyme is genetically engineered into two inactive fragments: an enzyme donor (ED) conjugated with the drug analog, and an enzyme acceptor (EA). When the two fragments associate, the full enzyme converts a substrate into a cleaved colored product. When there is no free drug present, the antibody inhibits the fragments from associating by binding with the ED fragment. If free drug is present, the free drug will compete with the ED fragment in solution to bind to the antibody. With the free drug competing for the limited antibody sites, the ED fragment and EA fragments can associate, thus produced the cleaved colored product, which will generate a colorimetric signal, which can be measured at the 572 nm wavelength. The colorimetric signal intensity is directly proportional to the amount of free drug present.

2.2 Assess Detection at Decision Point

The limit of detection was evaluated by preparing each analyte separately at 20, 50, 100, and 1000 ng/mL in urine. Samples were analyzed at both cutoff concentrations to determine if the analyte could be detected at one or both cutoff(s). Analytes not detected at 1000 ng/mL for a cutoff were considered not detectable. If detected, the appropriate concentration was used as the decision point to determine the precision at the immunoassay's cutoff.

2.3 Assess Precision at Decision Point

Following AAFS Standards Board Standard 036,[15] Standard Practices for Method Validation in Forensic Toxicology, precision was assessed. Three QC pools of the analyte were prepared at -50%(QCN), the decision point, and +100%(QCP), which were analyzed in five different runs (n=3)

along with the respective immunoassay's control materials. The total mean (n=15), standard deviation (SD), and percent coefficient of variation (%CV) were calculated for each QC concentration. A decision point was considered valid if the %CV for the QC was ≤20% for each concentration, and the total mean of the QCN and QCP ±2SD did not overlap the mean of the decision point.

3 RESEARCH RESULTS & DISCUSSION

3.1 Assay Verification

The six commercially available liquid enzymatic immunoassay methods for the detection of cannabinoids (HEIC) in urine at the 50 ng/mL (federal) and 20 or 25 ng/mL (lower) screening cutoff concentrations were setup and verified successfully on the Abbott Architect Plus c4000.

The ABBOTT, CEDIA, DRI, and LZI were successfully setup and verified utilizing the manufacturer guidelines. For this reason, precision and accuracy for these assays was only assessed for three days instead of five days.

The ROCHE assay parameters had to be slightly modified from manufacturer guidelines in order to be compatible with the Abbott Architect Plus c4000. Since the ROCHE assay is a turbidimetric assay, the absorbance for the lattice generated fell outside the max absorbance reading for the Abbott Architect Plus c4000, thus resulting in a failed calibration. In order to bring the max absorbance down to an acceptable reading range for the Abbott Architect Plus c4000, the R1 reagent was diluted with water (45 µL). The minimum allowed diluting amount for R1 is 45 µL, therefore, this amount was picked. With this change, the ROCHE assay was able to be successfully calibrated. Precision and accuracy were assessed for five days due to the parameter modification.

The SYVA assay parameters were slightly modified from manufacturer guidelines in order to be compatible with the Abbott Architect Plus c4000. The Abbott Architect Plus c4000 requires the total volume for the sample, R1, and R2 reagent to fall within the range of 160 µL to 360 µL. According to manufacturer guidelines for the SYVA assay, the total volume for the sample, R1, and R2 reagent fell just outside the required minimum volume at 150 µL. In order to correct this, the manufacturer amounts of the sample, R1, and R2 reagents were increased by a constant factor of 1/15 in order to bring the total volume up to the acceptable range for the Abbott Architect Plus c4000. This did not change the ratios set by the manufacturer for the sample, R1, and R2 reagent. With this change, the SYVA assay was able to be successfully calibrated. Precision and accuracy were assessed for five days due to the parameter modification.

All relevant information regarding assay verification can be found in Appendix A.

3.2 Δ8-THC & Metabolites

Δ8-THC and its metabolites 11-OH-Δ8-THC and 11-COOH-Δ8-THC were assessed for detectability. The results for each analyte are summarized, below.

3.2.1 Δ8-THC

The minimum detectable concentration for Δ8-THC was 200 ng/mL using the 50 ng/mL cutoff by Abbott, DRI, LZI, and SYVA. The minimum detectable concentration for Δ8-THC was 100 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), DRI(20), LZI(25), and SYVA(20). Δ8-THC was not detected at either cutoff by CEDIA or ROCHE. All relevant information regarding Δ8-THC detectability can be found in Table 1 and 2 in Appendix B.

3.2.2 11-OH-Δ8-THC

The minimum detectable concentration for 11-OH-Δ8-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, ROCHE, and SYVA. The minimum detectable concentration for 11-OH-Δ8-THC was 50 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), ROCHE(20), and SYVA(20). All relevant information regarding 11-OH-Δ8-THC detectability can be found in Table 3 and 4 in Appendix B.

3.2.3 11-COOH-Δ8-THC

The minimum detectable concentration for 11-COOH-Δ8-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, ROCHE, and SYVA. The minimum detectable concentration for 11-COOH-Δ8-THC was 50 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), ROCHE(20), and SYVA(20). All relevant information regarding 11-COOH-Δ8-THC detectability can be found in Table 5 and 6 in Appendix B.

3.3 CBD & Metabolites

CBD and its metabolites 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD were assessed for detectability. Abn-CBD and CBDA-A were also assessed for detectability. The results for each analyte are summarized below.

3.3.1 6-OH-CBD

6-OH-CBD was not detected by any of the six immunoassays at the 50 ng/mL cutoff. The minimum detectable concentration for 6-OH-CBD was 1000 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), DRI(20), LZI(25), and ROCH(20). All relevant information regarding 6-OH-CBD detectability can be found in Table 1 in Appendix C.

3.3.2 7-OH-CBD

7-OH-CBD was not detected by any of the six immunoassays at the 50 ng/mL cutoff. The minimum detectable concentration for 7-OH-CBD was 1000 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), DRI(20), and LZI(25). All relevant information regarding 7-OH-CBD detectability can be found in Table 2 in Appendix C.

3.3.3 CBD, 7-COOH-CBD, Abn-CBD, & CBDA-A

CBD, 7-COOH-CBD, Abn-CBD, and CBDA-A were not detected by any of the six immunoassays at either the 50 ng/mL or 20 or 25 ng/mL cutoffs. All relevant information regarding CBD, 7-COOH-CBD, Abn-CBD, and CBDA-A detectability can be found in Table 3 in Appendix C.

3.4 Δ10-THC

The four chiral Δ10-THC analogs 9(R)-$\Delta^{6a,10a}$-THC, 9(S)-$\Delta^{6a,10a}$-THC, (6aR,9R)-Δ^{10}-THC, and (6aR,9S)-Δ^{10}-THC were assessed for detectability. The results for each analyte are summarized below.

3.4.1 9(R)-$\Delta^{6a,10a}$-THC

The minimum detectable concentration for 9(R)-$\Delta^{6a,10a}$-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, and SYVA. The minimum detectable concentration for 9(R)-$\Delta^{6a,10a}$-THC was 50 ng/mL using the 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), and SYVA(20). 9(R)-$\Delta^{6a,10a}$-THC was not detected by the ROCHE screening kit at either cutoff. All relevant information regarding 9(R)-$\Delta^{6a,10a}$-THC detectability can be found in Table 1 and 2 in Appendix D.

3.4.2 9(S)-$\Delta^{6a,10a}$-THC

The minimum detectable concentration for 9(S)-$\Delta^{6a,10a}$-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, and SYVA. The minimum detectable concentration for 9(S)-$\Delta^{6a,10a}$-THC was 50 ng/mL using 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), and SYVA(20). 9(S)-$\Delta^{6a,10a}$-THC was not detected by the ROCHE screening kit at either cutoff. All relevant information regarding 9(S)-$\Delta^{6a,10a}$-THC detectability can be found in Table 3 and 4 in Appendix D.

3.4.3 (6aR,9R)-Δ^{10}-THC

The minimum detectable concentration for (6aR,9R)-Δ^{10}-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, and SYVA. The minimum detectable concentration for (6aR,9R)-Δ^{10}-THC was 50 ng/mL using the 20 or 25ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), and SYVA(20). (6aR,9R)-Δ^{10}-THC was not detected by the ROCHE screening kit at either cutoff. All relevant information regarding (6aR,9R)-Δ^{10}-THC detectability can be found in Table 5 and 6 in Appendix D.

3.4.4 (6aR,9S)-Δ^{10}-THC

The minimum detectable concentration for (6aR,9S)-Δ^{10}-THC was 100 ng/mL using the 50 ng/mL cutoff by Abbott, CEDIA, DRI, LZI, and SYVA. The minimum detectable concentration for (6aR,9S)-Δ^{10}-THC was 50 ng/mL using 20 or 25 ng/mL cutoff by Abbott(20), CEDIA(25), DRI(20), LZI(25), and SYVA(20). (6aR,9S)-Δ^{10}-THC was not detected by the ROCHE screening kit at either cutoff. All relevant information regarding (6aR,9S)-Δ^{10}-THC detectability can be found in Table 7 and 8 in Appendix D.

3.5 Olivetol & Metabolite

Olivetol and its metabolite olivetolic acid were assessed for detectability. The results for each analyte are summarized below.

3.5.1 Olivetol

Olivetol was only detected at 1000 ng/mL by the ROCHE screening kit at both the 50 ng/mL and 20 or 25 ng/mL cutoffs. The initial %CV -50%(QCN) for the 50 ng/mL was 29%, which exceeds the acceptable ≤20% guideline. To rectify, additional days of precision were planned. However, during this process, the initial lot of ROCHE reagent ran out. Upon purchasing more reagent, it was noted that the reagent was a new LOT. Upon running the olivetol analyte with the new reagent, it was observed that olivetol did not cross-react with the new lot. All relevant information regarding olivetol detectability can be found in Table 1 and 2 in Appendix E.

3.5.2 Olivetolic Acid

Olivetolic acid was not detected by any of the six immunoassays at either the 50 ng/mL or 20 or 25 ng/mL cutoffs. All relevant information regarding olivetolic acid detectability can be found in Table 3 in Appendix E.

4 Conclusion

The six commercially available homogeneous urine cannabinoid screening kits were able to detect Δ8-THC, 11-OH-Δ8-THC, 11-COOH-Δ8-THC, 6-OH-CBD, 7-OH-CBD, all Δ10-THC chiral analogs, and olivetol with varying selectivity depending on the screening kit. The six commercially available homogeneous urine cannabinoid screening kits were not able to detect CBD, 7-COOH-CBD, Abn-CBD, CBDA-A, or olivetolic acid. For those kits that were able to

detect Δ8-THC and its metabolites, the limit of detection was ~4 times the assay's cutoff. For those kits that were able to detect Δ10-THC, the limit of detection was ~4 times the assay's cutoff.

The results of this project will increase the knowledge of the forensic science community by increasing the basic understanding and capabilities of current homogeneous cannabinoid immunoassay screening assays to detect the increasing number of THC analogs being encountered. This will assist the forensic science and regulatory community understand the ability of routine urine cannabinoid drug testing to detect these analogs and what potential interference they may have on cannabinoid testing results.

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EDUCATION

Virginia Commonwealth University – Richmond, VA *August 2021 – Expected May 2023*
M.S. in Forensic Science – Concentration in Forensic Chemistry/Drugs and Toxicology
Rutgers University – New Brunswick, NJ *August 2017 – May 2021*
B.A. in Chemistry & B.A. in Criminal Justice

TECHNICAL SKILLS

EDXRF, FT-NMR, FT-IR, GC, GC-MS, ICP-OES, LC-MS-MS, UV-Vis, HPLC, Fluorescence, Affinity Chromatography, Ion Chromatography, Molecular Biology, PCR, Plasmid Purification, DNA Gel Electrophoresis, DNA Sequence Analysis, Protein Expression/Purification, Protein Gel Electrophoresis, Protein Analysis, Immunoassay Analysis

RELEVANT COURSES

Organic Chemistry, Organic Chemistry Lab 1 & 2, Inorganic Chemistry, Biochemistry, Analytical Chemistry Lab, Instrumental Analysis Lab, Instrumentation in Forensic Chemistry, Advanced Drug Analysis Lab, Analytical Considerations in Forensic Toxicology Lab, Applications in Forensic Toxicology Lab, Drug Dependence, Forensic Medicine

ACADEMIC RESEARCH

FIRM Specialty Testing Laboratory at VCU Health
Graduate Research Assistant *January 2022 – Present*

• Project: Evaluating the Ability to Detect Cannabinoid Analogs & Their Metabolites Utilizing Commercially Available HEICSs – funded in part by the National Institute of Justice (15PNIJ-21-GG-04188-RES).

- Set up and verified six commercially available HEICs according to manufacturer standards at the federal (50 ng/mL) and lower (20 or 25ng/mL) screening cutoff concentrations.
- Assessed Delta 8–THC, Olivetol, CBD, and Delta–10, their major metabolites (except Delta–10) in urine for detectability following AAFS Standards Board Standard 036, Standard Practices for Method Validation in Forensic Toxicology.
- Currently working on characterizing Delta 10–THC's metabolites as they are not commercially available. After characterization, the metabolites will be assessed in urine for detectability.

Gene Hall Research Group – Wright-Rieman Laboratories, Rutgers University
Undergraduate Research Assistant *September 2020 – June 2021*

• Project: Forensic Investigation of Adulterated Dietary Supplements

- Utilized ATEEM-Spectroscopy to build a library of fluorescence excitation-emission matrices of dietary supplements marketed as containing omega-3 and omega-7 fatty acids.
- Utilized Parallel Factor Analysis (PARAFAC) to group dietary supplements depending on the presence or absence of natural omega-3 and omega-7 fatty acids.

Khare Lab – Center for Integrative Proteomics Research, Rutgers University
Undergraduate Research Assistant *September 2018 – August 2020*

• Project: 2nd Layer Split PPV AND Gate *June 2019 – August 2020*

- Optimized split Plum Pox Virus (PPV) protease-based constructs by fusing constructs with MBP via molecular biology for use in an nth layer AND gate.
- Expressed constructs using the BL-21 (DE3) cell line in autoinduction media ZYP-5052.
- Worked on optimizing the purification protocol to reduce truncation products by testing a standard Ni-NTA purification versus an amylose purification.
- Demonstrated "ON" and "OFF" switchability via a fluorescence-based assay.

Project: Improving Existing XOR Protease Logic Switch *September 2018 – May 2019*

- Optimized the Soybean Mosaic Virus (SbMV) Protease Sarc Homology 2 (SH2) Phosphorylation based logic switch (with Tobacco Etch Virus (TEV) inhibition) developed as a component of an XOR Gate by fusing constructs with a Maltose Binding Protein (MBP) via molecular biology.
- Expressed constructs using the BL-21 (DE3) cell line in autoinduction media ZYP-5052 and purified them using a standard Ni-NTA purification.
- Demonstrated TEV cleavage at the designed cutsites by incubating purified constructs with TEV fast protease.
- Demonstrated "ON" and "OFF" switchability via phosphorylation in a fluorescence-based assay.

WORK EXPERIENCE

Chemistry Laboratory Teaching Assistant, VCU *August 2021 – Present*

- Teaching maximum capacity (24 students) undergraduate Organic Chemistry 1 & 2 Laboratories.
- Performed all assistant teaching duties including: teaching/facilitating laboratory experiments, mentoring students individually, grading lab reports, and proctoring exams.

Center for Integrative Proteomics Research, Rutgers University *September 2017 – May 2021*

- Maintained a smoothly functioning lab by monitoring inventory, assisting with placing restocking orders, maintaining general lab upkeep, and preparing commonly used buffers/reagents.
- Trained new undergraduate students on various protocols and instruments that are commonly used.

SCHOLARSHIPS

Graduate Research Assistant Award *Fall 2022*

- Provided full out-of-state university tuition coverage.

Dr. Teri Stockham Scholarship in Forensic Toxicology *Spring 2022*

- Provided financial support to a graduate student pursuing a Master of Science in Forensic Science with a concentration in forensic chemistry/drugs and toxicology.

Graduate School Master's Scholarship *Spring 2022*

- Provided in-state university tuition for underrepresented populations pursuing a master's degree.

ACCEPTED ABSTRACTS/PRESENTATIONS

- **Pokhai AA**, Poklis JL, Wolf CE, Williams GR, *Assessing the Limit of Linearity of Cannabinoid Analogs (Δ8-THC, Δ10-THC, and CBD) and their Major Metabolites in Six Commercial Homogeneous Cannabinoid Urine Screening Kits*. 61st Annual Eastern Analytical Symposium. Plainsboro, NJ. November 2022.
- **Pokhai AA**, Poklis JL, Wolf CE, Williams GR, "*Diet-Weed's" (Δ8-THC) Cross-Reactivity with Six Commercial Cannabinoid Urine Screening Kits*, 52nd Annual Meeting, Society of Forensic Toxicologists. Cleveland, Ohio. October 2022.
- Wolf CE, Holt AK, Karin KN, **Pokhai AA**, Poklis JL, Williams GR, *The Potential to Detect 13 Cannabinoid Acetate Analogs in Urine Using 6 Commercially Available Cannabinoid Homogeneous Screening Kits*. 52nd Annual Meeting, Society of Forensic Toxicologist. Cleveland, Ohio. October 2022.
- **Pokhai AA,** *Assessing the Detectability of Cannabinoid Analogs (Delta-8 THC, Delta-10 THC and CBD) and their Major Metabolites in Six Commercial Cannabinoid Urine Screening Kits*. Master's Thesis Defense. Virginia Commonwealth University, Richmond, Virginia. October 2022.
- **Pokhai AA**, Hall GS, *Unique Absorbance and Fluorescence Fingerprints of Dietary Supplements Revealed by A-TEEM Spectroscopy*, 60th Annual Eastern Analytical Symposium. Plainsboro, NJ. November 2021.

- **Pokhai AA**, Hall GS, *Unique Absorbance and Fluorescence Fingerprints of Dietary Supplements Revealed by A-TEEM Spectroscopy*. Eastern Analytical Symposium - September Virtual Student Symposium. Virtual. September 2021.
- **Pokhai AA,** Hall GS, *Fluorescence Excitation and Emission (EEM) Spectra Coupled with PARAFAC for Characterization and Classification of Dietary Supplements*. Rutgers Undergraduate Chemistry Research Symposium. Virtual. April 2021.
- **Pokhai AA,** Dolan EM, Khare SD, *Improving Protease Logic Switches with Solubilizing Protein Domains*. Rutgers Undergraduate Chemistry Research Symposium. Rutgers University, New Brunswick, NJ. April 2019.

APPENDIX A

Table 1. ABBOTT Assay Verification

	Low Control: C3		**Cutoff: 20 ng/mL**		**High Control: S1E**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT(20)	9	12	19	4	28	6
	Low Control: 40 ng/mL		**Cutoff: 50 ng/mL**		**High Control: 60 ng/mL**	
ABBOTT(50)	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
	38	2	50	2	66	1

Table 2. CEDIA Assay Verification

	Low Control: L25		**Cutoff: 25 ng/mL**		**High Control: H25**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
CEDIA(25)	15	4	26	5	46	4
	Low Control: L50		**Cutoff: 50 ng/mL**		**High Control: H50**	
CEDIA(50)	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
	27	5	52	6	79	6

Table 3. DRI Assay Verification

	Low Control: C3		**Cutoff: 20 ng/mL**		**High Control: S1E**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
DRI(20)	10	12	19	3	28	7
	Low Control: S1EE		**Cutoff: 50 ng/mL**		**High Control: S2**	
DRI(50)	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
	20	6	49	1	64	3

Table 4. LZI Assay Verification

	Low Control: 18.75 ng/mL		**Cutoff: 25 ng/mL**		**High Control: 31.25 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
LZI(25)	17	3	25	2	34	2
	Low Control: 37.5 ng/mL		**Cutoff: 50 ng/mL**		**High Control: 62. 5 ng/mL**	
LZI(50)	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
	37	5	51	1	58	1

Table 5. ROCHE Assay Verification

	Low Control: 15 ng/mL		**Cutoff: 20 ng/mL**		**High Control: 25 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ROCHE(20)	14	10	17	11	26	8
	Low Control: 37.5 ng/mL		**Cutoff: 50 ng/mL**		**High Control: 62.5 ng/mL**	
ROCHE(50)	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
	41	5	47	12	65	9

Table 6. SYVA Assay Verification

	Low Control: C3		**Cutoff: 20 ng/mL**		**High Control: S1E**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
SYVA(20)	12	4	20	2	27	7
	Low Control: S1E		**Cutoff: 50 ng/mL**		**High Control: S2**	
SYVA(50)	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
	18	10	50	1	65	5

APPENDIX B

Table 1. Δ8-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 100 ng/mL		DP: 200 ng/mL		+100%DP: 400 ng/mL	
	X̄	%CV	X̄	%CV	X̄	%CV
ABBOTT	30	23	77	11	92	3
CEDIA	---	---	---	---	---	---
DRI	30	15	77	10	91	4
LZI	27	14	53	5	58	2
ROCHE	---	---	---	---	---	---
SYVA	31	15	65	11	91	4

Table 2. Δ8-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		DP: 100 ng/mL		+100%DP: 200 ng/mL	
	X̄	%CV	X̄	%CV	X̄	%CV
ABBOTT(20)	15	23	39	17	100	8
CEDIA(25)	---	---	---	---	---	---
DRI(20)	18	27	43	19	109	10
LZI(25)	13	29	31	16	62	5
ROCHE(20)	---	---	---	---	---	---
SYVA(20)	15	20	32	10	56	5

Table 3. 11-OH-Δ8-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		DP: 100 ng/mL		+100%DP: 200 ng/mL	
	X̄	%CV	X̄	%CV	X̄	%CV
ABBOTT	19	28	55	14	95	1
CEDIA	18	29	56	20	101	4
DRI	20	27	54	16	93	1
LZI	22	25	49	7	58	0
ROCHE	36	36	64	14	115	109
SYVA	23	25	51	15	95	1

Table 4. 11-OH-Δ8-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 25 ng/mL		DP: 50 ng/mL		+100%DP: 100 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT(20)	9	47	24	26	69	15
CEDIA(25)	7	42	28	29	47	5
DRI(20)	9	46	29	26	76	18
LZI(25)	8	38	27	22	60	3
ROCHE(20)	14	43	28	20	53	19
SYVA(20)	9	36	29	22	59	8

Table 5. 11-COOH-Δ8-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		**DP: 100 ng/mL**		**+100%DP: 200 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT	22	27	65	14	94	1
CEDIA	23	24	107	14	126	4
DRI	21	20	65	17	93	1
LZI	26	25	55	1	58	1
ROCHE	46	24	88	12	189	6
SYVA	25	27	65	8	98	2

Table 6. 11-COOH-Δ8-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 25 ng/mL		**DP: 50 ng/mL**		**+100%DP: 100 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT(20)	10	30	29	29	84	15
CEDIA(25)	12	32	50	20	60	2
DRI(20)	10	36	29	23	93	17
LZI(25)	10	14	31	25	64	1
ROCHE(20)	22	17	44	24	91	7
SYVA(20)	13	12	34	22	64	1

*%CV is the ratio of the standard deviation to the mean. For analytes with an extremely low grand mean [0 - 5] an extremely high %CV is observed.

For analytes with a %CV that is slightly exceeded the acceptable ≤20% guideline, this can be rectified by running additional days of precision. However, due to limited reagent, this was not possible at the given time.

APPENDIX C

Table 1. 6-OH-CBD Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 500 ng/mL		**DP: 1000 ng/mL**		**+100%DP: 2000 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT	14	5	28	3	50	3
CEDIA	---	---	---	---	---	---
DRI	15	7	31	5	56	4
LZI	13	5	23	4	37	3
ROCHE	10	20	17	14	31	4
SYVA	---	---	---	---	---	---

*6-OH-CBD was not detected at the 50 ng/mL cutoff by any assay.

Table 2. 7-OH-CBD Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 500 ng/mL		**DP: 1000 ng/mL**		**+100%DP: 2000 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT(20)	8	11	20	7	42	3
CEDIA(25)	---	---	---	---	---	---
DRI(20)	9	13	23	7	48	4
LZI(25)	9	9	19	4	33	2
ROCHE(20)	---	---	---	---	---	---
SYVA(20)	---	---	---	---	---	---

*7-OH-CBD was not detected at the 50 ng/mL cutoff by any assay.

Table 3. CBD Analogs not Detected by Any Assay at Either Cutoff

Analyte	**Result**
CBD	Not detected at a minimum concentration of 1000 ng/mL by any assay at either cutoff.
7-COOH-CBD	
Abn-CBD	
CBDA-A	

APPENDIX D

Table 1. 9(R)-Δ6a,10a-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		DP: 100 ng/mL		+100%DP: 200 ng/mL	
	X̄	%CV	X̄	%CV	X̄	%CV
ABBOTT	12	14	40	9	86	2
CEDIA	9	19	32	16	64	6
DRI	13	15	45	17	87	4
LZI	11	10	33	15	54	4
ROCHE	---	---	---	---	---	---
SYVA	10	21	30	11	64	8

Table 2. 9(R)-Δ6a,10a-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 25 ng/mL		DP: 50 ng/mL		+100%DP: 100 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT(20)	4	113	16	18	52	9
CEDIA(25)	3	73	13	17	30	3
DRI(20)	4	99	18	17	64	15
LZI(25)	3	97	13	13	39	16
ROCHE(20)	---	---	---	---	---	---
SYVA(20)	3	92	12	14	35	12

Table 3. 9(S)-Δ6a,10a-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		DP: 100 ng/mL		+100%DP: 200 ng/mL	
	X̄	%CV	X̄	%CV	X̄	%CV
ABBOTT	12	18	38	9	88	4
CEDIA	10	20	42	15	93	5
DRI	12	14	42	15	89	3
LZI	12	17	34	15	56	3
ROCHE	---	---	---	---	---	---
SYVA	11	13	33	12	74	7

Table 4. 9(S)-Δ6a,10a-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 25 ng/mL		DP: 50 ng/mL		+100%DP: 100 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT(20)	2	92	15	19	50	9
CEDIA(25)	2	44	15	13	44	3
DRI(20)	3	68	18	11	59	12
LZI(25)	1	73	13	16	40	17
ROCHE(20)	---	---	---	---	---	---
SYVA(20)	2	56	13	11	37	11

Table 5. (6aR,9R)-Δ10-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		DP: 100 ng/mL		+100%DP: 200 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT	10	30	39	23	85	7
CEDIA	5	30	17	22	35	13
DRI	15	87	40	29	87	7
LZI	10	33	35	26	56	4
ROCHE	---	---	---	---	---	---
SYVA	11	24	39	26	81	11

Table 6. (6aR,9R)-Δ10-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 25 ng/mL		DP: 50 ng/mL		+100%DP: 100 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT(20)	3	66	14	30	50	22
CEDIA(25)	1	47	6	22	15	16
DRI(20)	3	76	16	22	57	27
LZI(25)	2	81	11	28	41	28
ROCHE(20)	---	---	---	---	---	---
SYVA(20)	4	58	14	22	41	19

Table 7. (6aR,9S)-Δ10-THC Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 50 ng/mL		DP: 100 ng/mL		+100%DP: 200 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT	10	46	32	17	67	18
CEDIA	1	68	5	18	8	11
DRI	9	42	32	21	63	6
LZI	6	42	21	23	40	6
ROCHE	---	---	---	---	---	---
SYVA	1	56	5	15	12	12

Table 8. (6aR,9S)-Δ10-THC Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 25 ng/mL		DP: 50 ng/mL		+100%DP: 100 ng/mL	
	X̄	%CV*	X̄	%CV	X̄	%CV
ABBOTT(20)	2	73	12	49	42	22
CEDIA(25)	0	123	1	54	4	14
DRI(20)	3	50	13	38	45	18
LZI(25)	1	114	7	47	24	22
ROCHE(20)	---	---	---	---	---	---
SYVA(20)	0	159	1	53	5	14

*%CV is the ratio of the standard deviation to the mean. For analytes with an extremely low grand mean [0 - 5] an extremely high %CV is observed.

For analytes with a %CV that is slightly exceeded the acceptable ≤20% guideline, this can be rectified by running additional days of precision. However, due to limited reagent, this was not possible at the given time.

APPENDIX E

Table 1. Olivetol Detectability at the 50 ng/mL Cutoff Concentration

	-50%DP: 500 ng/mL		**DP: 1000 ng/mL**		**+100%DP: 2000 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT	---	---	---	---	---	---
CEDIA	---	---	---	---	---	---
DRI	---	---	---	---	---	---
LZI	---	---	---	---	---	---
ROCHE	25	29	47	15	75	8
SYVA	---	---	---	---	---	---

*Olivetol did not cross-react with new LOT of ROCHE reagent.

Table 2. Olivetol Detectability at the 20 or 25 ng/mL Cutoff Concentration

	-50%DP: 500 ng/mL		**DP: 1000 ng/mL**		**+100%DP: 2000 ng/mL**	
	$\bar{X}$	%CV	$\bar{X}$	%CV	$\bar{X}$	%CV
ABBOTT(20)	---	---	---	---	---	---
CEDIA(25)	---	---	---	---	---	---
DRI(20)	---	---	---	---	---	---
LZI(25)	---	---	---	---	---	---
ROCHE(20)	23	16	38	12	63	11
SYVA(20)	---	---	---	---	---	---

*Olivetol did not cross-react with new LOT of ROCHE reagent.

Table 3. Olivetol Analog not Detected by Any Assay at Either Cutoff

Analyte	**Result**
Olivetolic Acid	Not detected at a minimum concentration of 1000 ng/mL by any assay at either cutoff.