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The Cross-Reactivity of the Cannabinoid Analogs (delta-8-THC, delta-10-THC and CBD) and their metabolites in Urine of Six Commercially Available Homogeneous Immunoassays.

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Abstract

There has been an exponential surge in the presence and use of cannabinoids since the federal legalization of hemp (Agricultural Improvement Act of 2018). This growth is not only attributed to delta-9-tetrahydrocannabinol (delta-9-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 delta-9-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 cannabinoid homogeneous immunoassay screening methods may be able to detect these emerging cannabinoid analogs and their metabolites.

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). Delta-8-THC, CBD, olivetol, and their major metabolites, and delta-10-THC chiral analogs were evaluated. The limit of detection was evaluated by preparing each analog at 20, 50, 100, and 1000 ng/mL in urine. Analytes did not cross react 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 and assess the linear range of the analog.

The six commercially available urine cannabinoid homogeneous immunoassay screening kits cross reacted with the following analogs: delta-8-THC, 11-OH-delta-8-THC, 11-COOH-delta-8-THC, 6-OH-CBD, 7-OH-CBD, all delta-10-THC chiral analogs, and olivetol with varying selectivity depending on the screening kit and cutoff concentration. The kits did not cross react with the following analogs: CBD, 7-COOH-CBD, Abnormal CBD, CBDA-A, and olivetolic acid.

Introduction

With the recreational use of cannabis legalized in 17 states, the District of Columbia, and the Northern Mariana Islands; decriminalization in 13 states, Guam, and the U.S. Virgin Islands; and the passage of the Agricultural Improvement Act of 2018, which federally legalized hemp, the presence and use of cannabinoids has seen an exponential growth. The increase in use has included not only tetrahydrocannabinol (delta-9-THC) and cannabidiol (CBD), the most abundant phytocannabinoid components of cannabis and hemp, respectively, but many other emerging psychoactive THC analogs. Recently, two analogs have become readily available on the commercial market, delta-8-tetrahydrocannabinol (delta-8-THC) and delta-10 tetrahydrocannabinol (delta-10-THC). These compounds can be found in various products including liquids used in electronic cigarettes, edibles, powders, etc. These products can be purchased via the internet or in local retail shops such as gas stations, vaping shops, and/or other businesses selling drug paraphernalia.

Federal regulation of cannabinoids and products is limited to the US Drug Enforcement Administration (DEA), which has classified marijuana as a Schedule 1 substance, and the US Food and Drug Administration (FDA), which has an approved formulation of THC (Marinol®)

and CBD (Epidiolex®). At present, both delta-8-THC and delta-10-THC are considered "legal" by many manufacturers, retail outlets, and state agencies. Based on their psychoactivity both delta-8-THC and delta-10-THC have a potential for abuse.

Delta-9-THC, CBD, delta-8-THC, and delta-10-THC are constitutional isomers ($C_{21}H_{30}O_2$, Molecular Weight (MW) 314), as are their expected carboxylic acid metabolites ($C_{21}H_{28}O_4$, MW 344). The THC constitutional isomers bind to and activate the CB1 cannabinoid receptor. Limited studies have shown that delta-8-THC is slightly less potent than THC in humans. Very little information is available about the potency of delta-10-THC. Because delta-8-THC may be extracted from hemp (legal) or synthesized from hemp-derived CBD (illicit), distinguishing the source of delta-8-THC is currently not possible, even if delta-8-THC exits only as a minor constituent of cannabis and hemp. The inability to definitely define delta-8-THC's source has created a substantial ambiguity regarding its legal status, and allowing for a plethora of commercial sources which can be obtained easily. Although some states have restricted its sale to consumers (Alaska, Arkansas, Arizona, Colorado, Delaware, Idaho, Iowa, Mississippi, Montana, Rhode Island, and Utah), many states have yet to regulate this cannabinoid. The prevalence of delta-8-THC in brick-and-mortar vape shops and online stores is a result of its psychoactive effects and regulatory ambiguity. A recent publication indicated that seized materials from DUI traffic stops have occasionally (3.7%) tested positive for an interfering chromatographic peak which was later identified as delta-8-THC (1). This is forensically significant because a non-scheduled drug (delta-8-THC) may result in a false positive, delta-9-THC, which can be prejudicial at a minimum but more likely have detrimental legal ramifications.

The increased presence of CBD and emerging THC analogs presents problem for forensic workplace, human performance, court ordered (i.e., probation/parole, incarceration, and child custody), US military, US Department of Transportation, etc. urine drug testing. Most urine drug testing 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). In regulated urine drug testing, screening is the initial test protocol. Relatively inexpensive and fast screening techniques are used to eliminate the vast number of negative specimens from those that require further costly confirmatory testing. Delta-9-THC carboxylate (delta-9-THC-COOH), the predominant THC metabolite, has been the standard compound in cannabinoid/marijuana abuse testing. In 1994, federal regulations lowered the screening cutoff for delta-9-THC-COOH to 50 ng/mL from 100 ng/mL in urine to increase the detection window for potential THC abuse. (3,4) In some scenarios, 20 or 25 ng/mL is used as the cutoff to extend the detection window. Little published information is known of cross-reactivity of THC analogs (5) with commercial urine drug testing kits, and little more is known of the analog's urine metabolites cross-reactivity. (6,7) Most publications on THC analog testing involve liquid chromatography coupled to mass spectrometry, which is not always cost effective or the timeliest screening technique. Many urine drug testing laboratories screening for marijuana/THC using liquid homogeneous immunoassay cannabinoid (HEIC) methods. These methods are preferred as they require no pretreatment of the urine samples; these methods are adaptable to automated immunoassay analyzers, which means minimal skill is required to perform the testing, and they are fast. Depending on the speed of the analyzer, hundreds to thousands of tests can be performed in an hour.

Most urine drug testing follows some type of federally mandated regulations. Under the Federal Agency Workplace Drug Testing Programs , the Medical Review Officer Manual (8), delta-9-

THC-COOH is the only cannabinoid tested and to report a positive urine specimen, “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.”
At present little is known as to capabilities of homogenous immunoassays to detect the current commercially available cannabinoids; the potential for these emerging analogs and/or their metabolites to cause false positive screening results could be high. This may lead to a similar situation that occurred in December 1998, where 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.” (2,8,9)
CBD, delta-8-THC, and delta-10-THC and their metabolites have a similar chemical structure to delta-9-THC and its major metabolites. These similarities present potential problems with the current immunoassay screening methods, regularly employed in forensic, clinical, or pain management testing laboratories, which are used to screen for the presence of THC-COOH in urine. With the structural difference between delta-9-THC, delta-8-THC, and delta-10-THC being the location of one double-bond, there is a high degree of scientific probability that delta-8-THC and delta-10-THC could cross-react with available cannabinoid/THC immunoassays. Elucidating the nature of this interaction would be of aid to forensic laboratories and anyone else involved in urine cannabinoid testing and would allow for better understanding as to the potential false-positive THC screens rate that can result in a costly increase in the number of confirmatory tests. The increasing commercial availability of delta-8-THC fortified products indicated that this rise in cost may be substantial. Anecdotal evidence from various forensic laboratories including our own suggests that some HEICs have the potential to cross-reactive with CBD, delta-8-THC, delta-10-THC, and/or their metabolites. As such, the cross-reactivity of 6 HEICs test kits, Abbott Cannabinoids, CEDIA™ THC and DRI® Cannabinoid Assays, LZI Cannabinoids (cTHC) Enzyme), ONLINE DAT Cannabinoid II and Syva EMIT®II Plus were evaluated using delta-8-THC, 11-Hydroxy-delta-8-tetrahydrocannabinol (11-OH-delta-8-THC), (−)-11-nor-9-carboxy-Δ^8-THC (11-COOH-delta-8-THC), CBD, 6α-OH Cannabidiol (6-OH-CBD), 7-Hydroxycannabidiol (7-OH-CBD), 7-Carboxy cannabidiol (7-COOH-CBD), Abnormal CBD (Abn-CBD), Cannabidiolic acid (CBDA-A), 9(S)-Δ6a,10a-Tetrahydrocannabinol (9(R)-$\Delta^{6a,10a}$-THC), 9(R)-Δ6a,10a-Tetrahydrocannabinol (9(S)-$\Delta^{6a,10a}$-THC), (6aR,9R)-Δ10-THC, (6aR,9S)-Δ10-THC, olivetol, and olivetolic acid. Olivetol a precursor in various syntheses of THC and has been purported to dampen the high from an excessive cannabinoid dose and is also available commercially and may also potentially cross-react with HEICs. The chiral analogs were included in the study due to their commercial availability and also to evaluate the effect of chirality has on the analogs cross-reactivity.

Methods

Materials

The standards, delta-8-THC, 11-OH-delta-8-THC, 11-COOH-delta-8-THC, CBD, 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD, Abnormal CBD (Abn-CBD), CBDA-A, 9(R)-$\Delta^{6a,10a}$-THC, 9(S)-$\Delta^{6a,10a}$-THC, (6aR,9R)-Δ10-THC, (6aR,9S)-Δ10-THC, olivetol, and olivetolic acid were obtained from Cayman Chemical Company (Ann Arbor, MI). The standard, 7-COOH-CBD was obtained from Cerilliant® Analytical Reference Standards (Round Rock, TX). These standards were available as 1.0 mg/mL solutions. Individual working standards were subsequently prepared in

methanol at 100.0 mg/L and 10.0 mg/L. Drug free human urine was obtained from Utak Laboratories Inc. (Valencia, CA) and was verified by screening and an in-house definitive cannabinoid assay.
The 6 HEICs and available calibrator and quality control materials were obtained from their manufacturers, (Tradename – Manufacturer) Abbott Cannabinoids – Abbott Diagnostics (Abbott Park, IL), CEDIA™ THC and DRI® Cannabinoid Assays– Thermo Fisher Scientific (Houston, TX), LZI Cannabinoids (cTHC) Enzyme Immunoassay – Lin-Zhi International (Santa Clara, CA), ONLINE DAT Cannabinoid II – Roche Diagnostics (Indianapolis, IN), and Syva EMIT®II Plus – Siemens Healthineers (Malvern, PA).
Bio-Rad laboratories (Hercules, CA) screening controls (C2, S1E, S2) were obtained for use as control materials for assay verification and daily controls for assays, which did not have manufacturer available low cutoff controls.

Instrumental Analysis

The HEICs were analyzed using an Abbott Architect c4000 autoanalyzer (Abbott Diagnostics, Abbott Park, IL) using the manufacturer's parameters and procedures as provided in each kit. The HEICs were verified to meet manufacturer's and the regulated urine testing standards over 5 days at the federal cannabinoid screening cutoff (50 ng/mL THC-COOH) and the manufacturer's lower cutoff (20 ng/mL or 25 ng/mL THC-COOH) using Bio-Rad screening controls.

Sample Preparation

The cross-reactivity of an analog with the 6 HEICs was initially evaluated by preparing each analog individually at concentrations of 20, 50, 100, and 1000 ng/mL in drug-free urine. These samples were analyzed in singlicate for each HEIC manufacturer's 50 ng/mL cutoff concentration and the manufacturer's lower cutoff concentration (20 ng/mL or 25 ng/mL THC-COOH) to determine if the analog was detectable at one or both of the manufacturer's cutoff concentrations. If an analog, prepared at the 1000 ng/mL concentration, was not detected at an HEIC's cutoff concentration, the analog was noted as not detectable at that specific HEIC's cutoff concentration. If the analog was detected at a cutoff concentration, the lowest detection concentration was used as the decision point to determine the precision for that compound at the HEIC's cutoff concentration.

In order to compare an analog's cross-reactivity across HEICs, the same prepared concentration of the analog was used as the decision point for the cutoff concentration (50 ng/mL or lower) for all HEICs that the analog was detectable at that cutoff concentration.

Precision was assessed by preparing quality control samples (QCs) in triplicate of the analog of interest (Decision point, -50 % (QCN) and + 50% (QCP)) using three different lots of QCs. The QCs were prepared daily, as a result of analog instability that was observed. These QCs pools were analyzed on five different times (n=3 per QC pool x 5 runs) along with daily HEIC's calibration and control materials. The total mean (n=15) for each QC concentration was calculated following AAFS Standards Board Standard 036, Standard Practices for Method Validation in Forensic Toxicology (10).

The linear response of detectable analogs was assess by preparing samples of the analog of interest at concentrations ranging from 0, 25, 50, 75, 100, 125, 150, 175, 200, 300, and 300 percent of the decision point and analyzed as singlicate.

Results and Discussion

The presented method demonstrates that many of the delta-8-THC analogs and delta-10-THC analogs cross-react with commercially available immunoassays. Also, some analogs do not cross-react with these immunoassays.

The Abbott assay at the 50 ng/mL cutoff cross reacted with delta-8-THC at 200 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 100 ng/mL. The Abbott assay at the 20 ng/mL cutoff cross reacted with delta-8-THC at 100 ng/mL, 6-OH-CBD and 7-OH-CBD at 1000 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 50 ng/mL. (Table 1)

The CEDIA assay at the 50 ng/mL cutoff cross reacted with delta-8-THC at 200 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 100 ng/mL The CEDIA assay at 25 ng/mL cross reacted with 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 50 ng/mL. (Table 2) The CEDIA assay did not cross reacted with delta-8-THC at either cutoff concentration.

The DRI assay at the 50 ng/mL cutoff cross reacted with delta-8-THC at 200 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 100 ng/mL. The DRI assay at the 20 ng/mL cutoff cross reacted with delta-8-THC at 100 ng/mL, 6-OH-CBD and 7-OH-CBD at 1000 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 50 ng/mL. (Table 3)

The Lin-Zhi assay at the 50 ng/mL cutoff cross reacted with delta-8-THC at 200 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 100 ng/mL. The Lin-Zhi assay at the 25 ng/mL cutoff cross reacted with delta-8-THC at 100 ng/mL, 6-OH-CBD and 7-OH-CBD at 1000 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 50 ng/mL. (Table 4)

The Roche assay at the 50 ng/mL cutoff cross reacted with delta-8-THC at 200 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 100 ng/mL The Roche assay at the 20 ng/mL cutoff cross reacted with 6-OH-CBD at 1000 ng/mL and 11-OH-delta-8-THC and 11-COOH-delta-8-THC at 50 ng/mL. (Table 5) The Roche assay at the 50 ng/mL and 20 ng/mL cutoff cross reacted with Olivetol at 1000 ng/ml. The Roche assay did not cross reacted with delta-8-THC or the four delta-10-THC analogs at either cutoff concentration.

The Syva assay at the 50 ng/mL cutoff cross reacted with delta-8-THC at 200 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 100 ng/mL. The Syva assay at the 20 ng/mL cutoff cross reacted with delta-8-THC at 100 ng/mL, and 11-OH-delta-8-THC, 11-COOH-delta-8-THC, and all four delta-10-THC chiral analogs at 50 ng/mL. (Table 6)

None of the six HEICs cross reacted with any CBD analog (CBD, 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD, Abn-CBD or CBDA-A) or Olivetolic acid at the 50 ng/mL cutoff. None of the six HEICs were able to detect CBD, 7-COOH-CBD, Abn-CBD, CBDA-A, or Olivetolic acid at the lower cutoffs (20 or 25 ng/mL).

Variable cross-reactivity was observed in the delta-10-THC analogs. The (6aR,9R)-Δ10-THC and (6aR,9S)-Δ10-THC analogs tended to have less cross-reactivity than the 9(R)-$\Delta^{6a,10a}$-THC and 9(S)-$\Delta^{6a,10a}$-THC analogs, with (6aR,9S)-Δ10-THC having the lowest cross-reactivity. Variable linearity response was observed between THC analog and HEIC. (Figure 1)

Conclusion

The six commercially available urine cannabinoid homogeneous immunoassay screening kits cross reacted with the following analogs: delta-8-THC, 11-OH-delta-8-THC, 11-COOH-delta-8-THC, 6-OH-CBD, 7-OH-CBD, all delta-10-THC chiral analogs, and olivetol with varying selectivity depending on the screening kit and cutoff concentration. The 6 HEICs kits did not cross react with the following analogs at the highest concentration tested, 1000 ng/mL: CBD, 7-COOH-CBD, Abnormal CBD, CBDA-A, and olivetolic acid. The observed linear response from the THC analogs was variable depending upon the analog and HEIC. The results of this study provide some insight into false positive screening results seen in urine THC drug testing when using a HEICs.

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