I. Introduction

The non-psychoactive cannabinoid, cannabidiol (CBD), has recently gained a reputation as a natural remedy for a wide variety of medical conditions. The sudden rise in CBD use has been propelled by the ability of the Toxicology Laboratory to definitively and selectively detect the use of marijuana. CBD products prepared and sold outside of the medical or legal marijuana market are generally unregulated. Many unregulated CBD products contain measurable amounts of tetrahydrocannabinol (THC), which is both the psychoactive compound in marijuana and the parent compound of the targeted analyte for drug testing. While the exposure to THC as a contaminant in CBD products is generally small, the exposure can be significant enough to result in a positive test for carboxy-THC. Inadvertent THC exposure from CBD products, similar to passive marijuana exposure, falls outside the intended goal of drug testing to serve as a deterrent to illicit drug use.

Objective: To develop a procedure to distinguish when positive carboxy-THC urine test results are from inadvertent exposure to contaminating THC from the use of CBD products versus THC exposure from the use of THC products.

II. Methods

Preparation of 7-COOH-CBD Standard

A commercial reference standard for 7-COOH-CBD was not available. 7-COOH-CBD was prepared from 11-COOH-CBD using the procedure outlined right, adapted from Djura, et al.

Urine Sample Analysis

Focusing was performed on 100 µL of urine. Analysis initiates with a sequential enzymatic and chemical hydrolysis of glucuronide metabolites of THC and CBD. Liberated metabolites were subsequently extracted with acetonitrile (ACN) and the organic extract was directly analyzed by LC-MS/MS.

LC-MS/MS analysis was performed on a Waters Acquity UPLC connected to a Scieix 6500+ MS. Chromatographic separation utilized an Acquity® HSS T3 column (50 x 2.1 mm, 1.8 µm) maintained at 50 °C using a flow rate of 0.65 mL/min and a mobile phase starting at 55% 10 mM ammonium acetate with 0.1% formic acid / 45% ACN and a mobile phase start at 55% 10 mM ammonium acetate with 0.1% formic acid / 45% ACN increasing in a step gradient reaching 95% ACN over 3 minutes. The following transitions were monitored in positive mode:

- THC & 7-COOH-CBD: 315.1 > 196.2, 323.1
- 11-COOH-THC & 7-COOH-CBD: 345.1 > 196.1, 291.1, 293.1, 187.1, 119.1 (11-COOH-THC), 354.3 > 196.1, 308.2

Quantitation was performed using a concurrently analyzed four-point calibration curve ranging from 0.6 – 300 ng/mL for THC and COOH, and 3.0 – 1500.0 ng/mL for 11-COOH-THC & 7-COOH-CBD.

V. Analysis of Samples from Users Claiming CBD Use

Over the course of 6 months, 436 samples were selected from a pain management setting where the donor claimed CBD use on their medication list. As knowledge that CBD use presents a conundrum for the laboratory testing industry, some users undoubtedly claim CBD use in an effort to obfuscate THC use. Other donors legitimately, exclusively use unregulated CBD. While still others intermix CBD with THC products. When testing using the described method, the ratio of the metabolites clearly fell into distinct subsets (see Figure 4 for an example data set). In one subset THC metabolites clearly dominated over CBD metabolites. These samples possessed a CBD to THC ratio of less than 1, indicating marijuana use. On the opposite extreme were samples where the ratio of THC was greater than 10; indicating CBD use whether or not the level of 11-COOH-THC exceeded a cutoff of 5 ng/mL. Also present was a group of samples where the ratio of THC to THC was greater than 10; indicating CBD use whether or not the level of 11-COOH-THC exceeded a cutoff of 5 ng/mL. 8% present was a group of samples where the ratio of THC to THC was greater than 10; indicating CBD use whether or not the level of 11-COOH-THC exceeded a cutoff of 5 ng/mL. Also present was an intermediate group with a metabolic ratio between 1 and 10. This intermediate group of samples may have come from donors consuming both THC and CBD or from users consuming heavily contaminated CBD products.

The developed assay protects CBD users from inadvertently positive THC tests resulting from low level THC contamination in commercial CBD products.

III. Testing of Commercial CBD Products

10 commercial CBD products (gummies, vapes, capsules, hemp flowers, etc.) were procured and tested to measure the content of CBD, THC, and their respective acidic precursors. Most products had no THC or THC levels less than 0.5% relative to CBD content. However, products with significant levels of THC are not uncommon.

The Impact on urine drug testing from contaminating levels of THC in CBD products is unknown.

IV. Analysis of Urine Samples from Known, Exclusive CBD Users

14 urine samples from persons known to use CBD but abstain from THC products were tested for CBD metabolites and THC metabolites (Figure 2). Six of those samples contained 11-COOH-THC and THC levels above 5 ng/mL. However, the ratio of the sum of CBD + 7-COOH-CBD relative to THC + 11-COOH-THC was generally greater than 10. Two samples were found to have ratios of less than 10, but the product used in one of those samples was unusually high in THC [10,66x]. The product associated with the other sample was not obtained for testing. A representative chromatogram from an exclusive CBD user is shown in Figure 3.

VI. Conclusion

Of the 436 samples from donors claiming CBD use, 17% were identified as marijuana users and 43% were CBD users. The remaining 40% fell into a group which we were able to calculate a ratio of CBD metabolites relative to THC metabolites, exclusive CBD users could be distinguished from THC users. Maximum CBD level: 1750 ng/mL, Maximum 7-COOH-CBD level: 2870 ng/mL, Maximum 11-COOH-THC level in a CBD user: 110 ng/mL (same patient had combined levels of CBDs of 2990 ng/mL).

References