National Laboratory Certification Program

DRUG TESTING MATTERS

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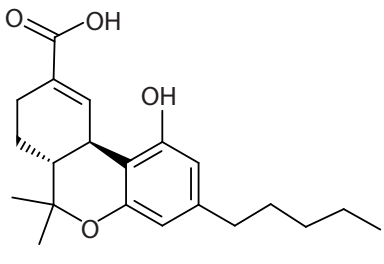
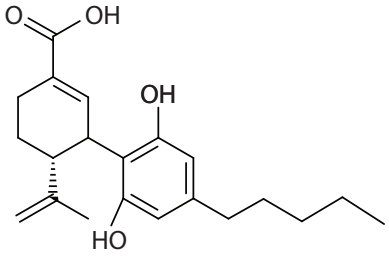
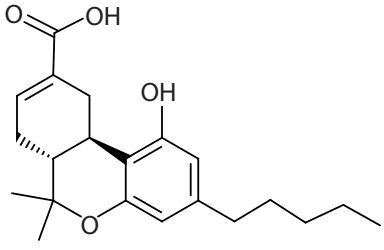
Cannabinoid Metabolites Pose Analytical Challenges in Urine Drug Testing Laboratories

During the last 40 years, laboratories engaged in testing urine specimens for the presence of cannabinoids have focused on the detection of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (Δ9-THCA), the urinary metabolite of Δ9-tetrahydrocannabinol (Δ9-THC). Given the emerging cannabis industry in the United States, two "new" compounds—7-carboxy-cannabidiol (CBDA) and 11-nor-9-carboxy-Δ8-tetrahydrocannabinol (Δ8-THCA)—have started to appear in specimens submitted to urine testing laboratories. The appearance of these two compounds could present analytical challenges to drug testing laboratories.

Figure 1. Cannabinoid Metabolites of Interest

		
Δ9-THCA	CBDA	Δ8-THCA

Cannabidiol (CBD) and its Metabolite, 7-Carboxy-Cannabidiol (CBDA)

Historically, cannabidiol (CBD), a non-intoxicating cannabinoid found in the *Cannabis sativa* plant, was among compounds that the U.S. Drug Enforcement Administration (DEA) referred to as "Marihuana" (DEA Code 7360), which includes all compounds found in the cannabis plant, and was listed as Schedule I under the Controlled Substances Act (CSA).[1] In 2016, CBD was included in a new class called "Marijuana Extract" (DEA Code 7350), also listed as Schedule I. In June 2018, the U.S. Food and Drug Administration (FDA) approved Epidiolex®, a pharmaceutical preparation of CBD extract (DEA Code 7367, Schedule V) used for the treatment of seizures associated with two rare and severe forms of epilepsy in children—Lennox-Gastaut syndrome and Dravet syndrome. Subsequently, the 2018 Agricultural Improvement Act (Farm Bill)[2] excluded hemp and hemp-derived products (e.g., CBD) from the CSA definition of marijuana, with the provision that the material contains less than 0.3% tetrahydrocannabinol (THC) on a dry weight basis. Hemp and hemp-derived products exceeding this THC limit are still classified as Schedule I drugs.

CBD also falls under the purview of the FDA. Currently, because of its use in an FDA-approved pharmaceutical preparation, CBD is not supposed to be sold as a dietary supplement. This means that over-the-counter ingestible CBD preparations are not legal in the eyes of the FDA. However, to date, the FDA has only issued warning letters to some manufacturers of CBD-containing supplements. This lack of enforcement regarding CBD has allowed an explosion of ingestible CBD products in the marketplace during the last 5 years, and that growth is continuing.

CBDA is one of the urinary metabolites detected after the ingestion of products containing CBD. As shown in Figure 1, CBDA is structurally analogous to Δ9-THCA, the primary urinary metabolite detected after the use of products containing Δ9-THC.

The analytical challenge that CBDA poses for drug testing laboratories lies in its ability to convert to Δ9-THCA and Δ8-THCA under acidic conditions. Previous reports have shown the conversion of CBD to Δ9-THC and Δ8-THC when samples are extracted and derivatized with acidic reagents, such as trifluoracetic anhydride with 1,1,1,3,3,3-hexafluoroisopropanol (TFAA-HFIP), pentafluoropropionic anhydride with 2,2,3,3,3-pentafluoro-1-propanol (PFPA-PFPOH), or pentafluoropropionic anhydride with 1,1,1,3,3,3-hexafluoroisopropanol (PFPA-HFIP).[3] The extent of this conversion has been reported to be as high as 84%.[3]

To test the conversion of CBDA to THCA, the National Laboratory Certification Program (NLCP) prepared a special set of proficiency test samples containing CBDA or THCA. The samples were submitted to the 25 laboratories accredited under the NLCP for THCA analysis using the laboratories' current initial and confirmatory methods (i.e., immunoassay and gas chromatography–mass spectrometry [GC-MS] or liquid chromatography–tandem mass spectrometry [LC-MS/MS]). Samples spiked with CBDA were prepared in urine at the following concentrations:

1. 25 ng/mL CBDA
2. 50 ng/mL CBDA
3. 100 ng/mL CBDA
4. 200 ng/mL CBDA
5. 500 ng/mL CBDA
6. 2500 ng/mL CBDA

Among the 25 laboratories, four different cannabinoids immunoassay kits were used: ThermoFisher DRI, Siemens EMIT II 5B3, Siemens EMIT II, and Roche KIMS. The cannabinoids immunoassay test results for the six samples were negative at all 25 laboratories, with reagent responses equivalent to that of a urine blank.

For confirmatory testing, 24 of the laboratories analyzed the samples by GC-MS with derivatization, and 1 laboratory analyzed the samples by LC-MS/MS without derivatization. For the laboratories using GC-MS, derivatizing agents included N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA), tert-butyldimethylsilyl chloride (TBDMS-Cl), n-propyl iodide (C_3H_7I), and methyl iodide (CH_3I). Of the 25 laboratories, 23 did not detect THCA in any of the CBDA-spiked samples. Using TBDMS-Cl, one laboratory reported THCA at 2.1 ng/mL in sample #5 (0.42% conversion) and at 5.7 ng/mL in sample #6 (0.23% conversion); using BSTFA, another laboratory reported THCA at 3.6 ng/mL in sample #6 (0.14% conversion). The conversions observed in these cases may have resulted from acidification of the aliquots during the extraction processes used in those laboratories.

The NLCP also requested that two laboratories (designated here as "Lab A" and "Lab B"), which had recently changed from acidic to non-acidic reagents, test the six samples using both their old and new reagents. Lab A had previously used PFPA-PFPOH, and Lab B had previously used PFPA-HFIP. The results of those laboratories' tests using their previous reagents are shown in Table 1.

Table 1. THCA Test Results for Samples Containing Only CBDA for Lab A (PFPA-PFPOH) and Lab B (PFPA-HFIP) with Percent Conversion Data

Sample No.	CBDA (ng/mL)	Lab A		Lab B	
		THCA (ng/mL)	Percent Conversion	THCA (ng/mL)	Percent Conversion
1	25	3.2	12.8%	34	136%
2	50	4.7	9.4%	76	152%
3	100	7.2	7.2%	151	151%
4	200	13.9	6.9%	327	163%
5	500	23.7	4.7%	737	147%
6	2,500	154.3	6.2%	3,798	152%

Both laboratories observed the conversion of CBDA to THCA during their sample preparation process. The high degree of conversion found by Lab B (136%–163%) was unexpected. This extreme degree of conversion might be explained as follows:

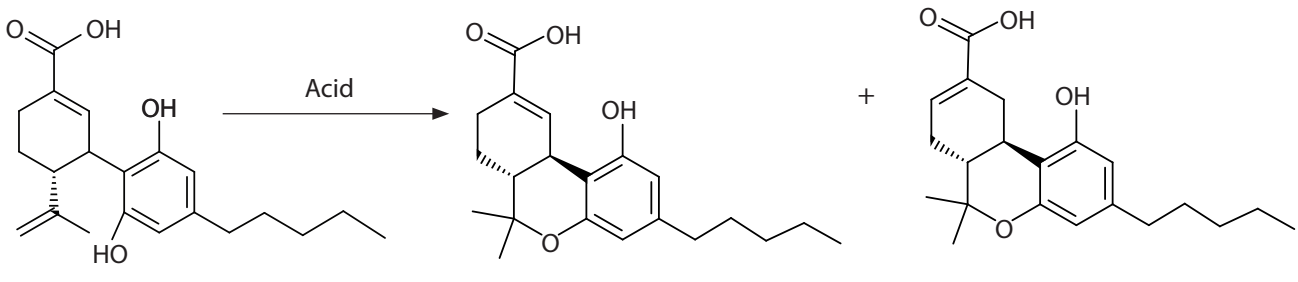
1. Lab B showed a 24% high bias for the THCA-spiked proficiency test samples included in the proficiency test set as "controls". This bias partially contributed to their apparent high conversion rate.
2. Preferential recovery of CBDA vs. deuterated THCA may have occurred before the derivatization step.

These two laboratories' results confirm that CBDA will convert to THCA when acidic derivatization reagents are used, in the same manner that CBD converts to THC in the presence of similar reagents. Furthermore, the conversion of CBDA to THCA is extremely high when HFIP is used with an acid anhydride, consistent with earlier reports.[3]

Based on these test results, laboratories should avoid the use of acidic derivatization reagents when testing urine samples for THCA.

Laboratories that wish to examine their analytical process for conversion of CBDA to THCA may want to obtain a sample of the compound for evaluation. Currently, CBDA is available from two sources.[4]

Figure 2. Conversion of CBDA under Acidic Conditions



CBDA Δ **9-THCA** **Δ8-THCA**

Δ8-Tetrahydrocannabinol and its Metabolite, 11-Nor-9-Carboxy-Δ8-Tetrahydrocannabinol (Δ8-THCA)

Δ8-THC is a compound found in low quantities in the *Cannabis sativa* plant. It is classified as an artifact compound formed by the degradation that occurs upon exposure to heat or light during processing and storage and results in a bond rearrangement in Δ9-THC. Its psychoactive potency is about half that of Δ9-THC, but this compound has been studied as an antiemetic in pediatric oncology[5] and as a potential anticancer compound.[6] There is at least one patent for the synthesis of Δ8-THC from CBD.[7] The patented synthesis forms Δ8-THC from CBD by treatment with Lewis acids. The compound can also be synthesized via the isomerization of Δ9-THC under acidic conditions. More recently, Δ8-THC has caught the attention of the cannabis industry, and a growing number of cannabis product manufacturers are now including Δ8-THC products in their offerings.

The urinary metabolite of Δ8-THC is Δ8-THCA. Because of the structural similarity between Δ8-THCA and Δ9-THCA (see Figure 1), urine testing laboratories might experience challenges in separating the two compounds via commonly used chromatographic methods. That is, Δ8-THCA might interfere with Δ9-THCA analysis, or the two compounds may co-elute, leading to the potential misidentification of Δ8-THCA as Δ9-THCA.

Previous reports have attributed interference in Δ9-THCA assays to Δ8-THCA.[8] Therefore, laboratories may wish to examine their confirmatory assay for the potential for interference from Δ8-THCA. At present, this compound is available from at least one supplier.[9]

Converging Cannabinoids

With the growing use of CBD and Δ8-THC products and the continuing use of Δ9-THC, laboratories may begin to see mixtures of all three compounds' metabolites in urine samples submitted for drug testing. Therefore, laboratories will likely need to update their confirmatory procedures to either eliminate CBDA and Δ8-THCA as interferences or to add the two compounds as new analytes to facilitate a more thorough review of their cannabinoids test results.

References

1. U.S. Department of Justice, DEA, Diversion Control Division, Drug & Chemical Evaluation Section. (2019). Lists of: Scheduling actions, controlled substances, regulated chemicals. Retrieved from https://www.deadiversion.usdoj.gov/schedules/orangebook/orangebook.pdf
2. Agriculture Improvement Act of 2018, Pub. L. No. 115-334, 132 Stat. 4490 (2018). https://www.congress.gov/115/plaws/publ334/PLAW-115publ334.pdf
3. Andrews, R., & Paterson, S. (2012). Production of identical retention times and mass spectra for Δ9-tetrahydrocannabinol and cannabidiol following derivatization with trifluoracetic anhydride with 1,1,1,3,3,3-hexafluoroisopropanol. Journal of Analytical Toxicology, 36, 61–65.
4. 7-Carboxy-cannabidiol (CAS Number: 63958-77-0) is available from Toronto Research Chemicals, North York, Ontario, Canada (https://www.trc-canada.com/) and from BDG Synthesis, Wellington, New Zealand (https://bdg.co.nz).
5. Abrahamov, A., Abrahamov, A., & Mechoulam, R., (1995). An efficient new cannabinoid antiemetic in pediatric oncology. Life Science, 56, 2097–2102
6. Munson, A. E., Harris, M. A., Dewey, W. L., & Carchman, R. A. (1975). Antineoplastic activity in cannabinoids. Journal of the National Cancer Institute, 55, 597–602.
7. Webster, G. R. B, Sarna, L. P., & Mechoulam, R. (2004). Conversion of CBD to delta-8-THC and delta-9-THC. United States Patent Application Publication, US 2004/0143126 A1.
8. Dawson, G. B., Njau, B., & Rana, S. (2019). Detection of delta-8-THC-COOH in urine samples and its implications in workplace drug testing. In The 57th Annual Meeting of the International Association of Forensic Toxicologists. Abstract ID 552, 177.
9. Δ8-THCA (CAS Number: 39690-06-7) is available from Organix, Inc., Woburn, MA (https://organixinc.com/).

Dale Hart has over 30 years of toxicology experience, including drugs of abuse testing in urine, oral fluid, and blood. Since 1998, he has worked as a Research Forensic Scientist in the Center for Forensic Sciences (CFS) at RTI International as a member of the Performance Testing (PT) Team for the National Laboratory Certification Program (NLCP) under contract with the U.S. Department of Health and Human Services (HHS). He is currently the NLCP PT Lead for Oral Fluid and Hair and also serves as an NLCP inspector. In addition to his work with the NLCP, he participates in CFS Research and Development projects and is the manager of RTI's Oral Fluid Proficiency Testing Program. Prior to joining RTI, Mr. Hart worked in forensic drug testing laboratories in the U.S. Military and in the private sector, including work at an HHS-certified laboratory as a laboratory manager and expert witness.