The Unseen Impact of Δ8-THC Products on the Regulated Drug Testing Industry

Jarod Kabulski, Sarah Small, Joseph McCrary, Kyle Bockenstedt, Steve Huffman, Richard Hewitt, Martin Jacques, Melissa Beals, David Kuntz; Clinical Reference Laboratory, Lenexa, Kansas

INTRODUCTION

In 2020, an unknown and unresolved chromatographic peak appearing in the Δ9-Carboxy-THC confirmation assay resulted in an increase in the reporting of specimens as invalid due to LC-MS/MS interference. The escalating occurrence of this undetermined interference initiated an investigation into the isolation and identification of the compound, as its retention time, parent ion, and product ions were the same as those for Δ9-Carboxy-THC. Through extensive chromatographic analysis and the re-validation of the Δ9-Carboxy-THC LC-MS/MS confirmation assay to provide compound resolution, it was possible to elucidate the existence of a once rarely seen metabolite, Δ8-Carboxy-THC, in regulated workplace drug test specimens.

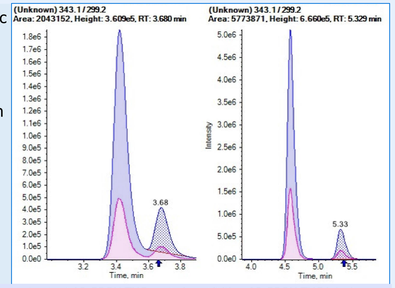


Figure A. Left: Sample chromatogram from standard Δ9-COOH-THC analysis exhibiting Δ8-COOH-THC interference; Right: Extended isocratic method with baseline separation of Δ8-COOH-THC and Δ9-COOH-THC.

OBJECTIVE

Use retrospective analysis of chromatography to determine the prevalence of Δ8-THC metabolite in federally-regulated (United States Department of Health and Human Services, United States Nuclear Regulatory Commission, and the United States Department of Transportation Administrations) urine drug test specimens.

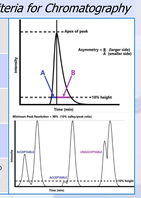
METHODS

Regulated urine drug test specimens having screened positive by immunoassay were analyzed by LC-MS/MS to confirm for the presence of Δ9-Carboxy-THC (Δ9-COOH-THC). If chromatographic interference was present in the confirmation data, samples affected were reanalyzed using a LC-MS/MS confirmation method that was developed to identify, quantitate, and separate Δ9-COOH-THC from Δ8-Carboxy-THC (Δ8-COOH-THC). These confirmation batches were manually examined for the presence of a Δ8-COOH-THC peak alongside the Δ9-COOH-THC peak in the chromatography.

The chromatographic review window allowed for observation of both Δ8-COOH-THC and Δ9-COOH-THC with baseline separation. For reporting purposes, only Δ9-COOH-THC was evaluated for quantitation, and peak acceptance was based on NLCP criteria. Finally, THC immunoassay results were compared to corresponding Δ9-COOH-THC LC-MS/MS results to determine screening and confirmation positivity rates.

Figure B: Acceptance Criteria for Chromatography

Relative Retention Time (RRT)	±2% of expected RRT of the analyte/internal standard pair established by the batch calibrator
Internal Standard (IS) Response	Total IS peak area = ≥10% of calibrator IS peak area
Symmetry / Peak Shape	Gaussian peaks; asymmetry at 10% of peak height = <3.0 for IS and quant peaks
Resolution	Adjacent peaks ≥90% resolved (≤ 10% valley/peak height ratio)
Ion Ratios (Qualifiers)	Ratio of abundance of quantitative to qualifier ion = ±20% of target ratio established by batch calibrator



Extraction Method

Samples were mixed with internal standard and hydrolyzed using 5N Potassium Hydroxide. Following hydrolysis, samples were neutralized with 5N Formic Acid and diluted with 0.1% Formic Acid in 50:50 DI H2O:Methanol.

Instrument Parameters

Table 1: UHPLC-MS/MS Parameters

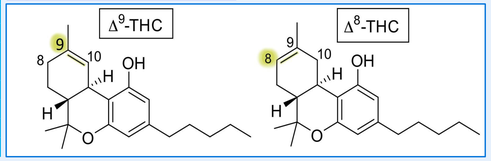
UHPLC System	Shimadzu Nexera	LC-30AD Pumps
		SIL-30 AC Auto Sampler
	Shimadzu Prominence	CBM-20A Controller
		CTO-20A Column Oven
		DGU-20A5 Degasser
Injection Volume	30 µL	
Analytical Column	Phenomenex Kinetex 2.6 µm C18 100 Å, 150 x 2.1 mm (Part No. 00F-4462-AN)	
Guard Column	Phenomenex SecurityGuard ULTRA Cartridge, UHPLC C18 2.1mm ID Column (Part No. AJ0-8782)	
Column Temp.	45°C	
Mobile Phase	Aqueous	10 mM Ammonium Formate
	Organic	50:50 Acetonitrile:Methanol
Flow Rate	0.600 mL/min	
Run Time	7.00 minutes	
Mass Spectrometer	Sciex API6500+ Triple Quad	
Ionization	Source Type:	Electrospray Ionization (ESI)
	Negative	
Source Temp.	650°C	
Data Analysis	MultiQuant by Sciex	

Table 2: Analyte Transition and Chromatographic Information

Analyte	Internal Standard	Precursor Ion	Product Ion Quantifier	Product Ion Qualifier	Elution Order Position
Δ9-COOH-THC	Δ9-COOH-THC-D9	343.1	299.2	245.1	Peak 3 (latest)
Δ9-COOH-THC-D9		352.1	308.2	254.1	Peak 2
Δ8-COOH-THC	n/a	343.1*	299.2*	245.1*	Peak 1 (earliest)

*These ions not monitored for Δ8-COOH-THC; method used for separation of compounds and quantitation of Δ9-COOH-THC only.

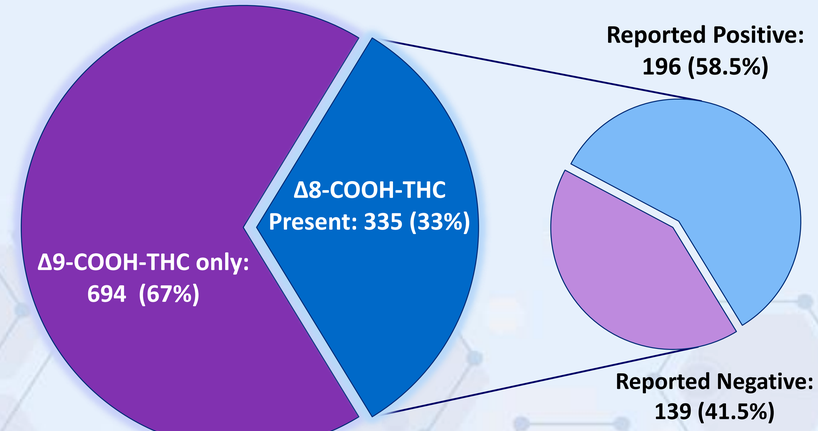
Figure C: Δ9-THC and Δ8-THC Structures



RESULTS / DISCUSSION

LC-MS/MS confirmation data was evaluated from April 2020 to May 2022. Although the immunoassay reagent in use remained constant throughout this period, the rate of screened-positive samples (50 ng/mL cutoff) to confirmed-positive samples (15 ng/mL cutoff) demonstrated a steady decline, just as availability of Δ8-THC products in stores and online was growing. In April of 2020, the Δ9-COOH-THC confirmation rate was 96%; that number had decreased to 78% by May of 2022. Further analysis incorporated creatinine levels in order to take state of hydration into consideration. In March of 2022, 417 urine samples screened positive for marijuana metabolite by immunoassay and confirmed negative by LC-MS/MS at the 15 ng/mL cutoff. The nonconfirming samples typically contained around 4 ng/mL of Δ9-COOH-THC when results were correlated across the normal creatinine range; therefore, data did not support excessive hydration as the cause of the reduced confirmation rate. Seven of the 417 nonconfirming samples did not contain Δ8-COOH-THC or Δ9-COOH-THC, most likely exhibiting immunoassay cross-reactivity due to Protonix or other unidentified cannabinoids.

Figure D. Left: Percentage of Δ8-COOH-THC confirmation samples containing Δ8-COOH-THC in one day; Right: Percentage of positive and negative reported results for samples displaying the presence of Δ8-COOH-THC



Recent re-review of LC-MS/MS chromatography was performed over a single day's regulated and non-regulated Δ9-COOH-THC confirmation batches (more than 1,000 specimens that had screened positive by immunoassay). Chromatography from 335 of these samples indicated the presence of Δ8-COOH-THC, constituting 33% of the day's total Δ9-COOH-THC confirmation workload. Of the 335 samples containing Δ8-COOH-THC, 41.5% reported negative (<15 ng/mL cutoff). By these numbers, it can be inferred that more than 10% of positive marijuana metabolite immunoassay screens on any given day may be attributed to the use of Δ8-THC.

Table 3: Increase in Presence of Δ8-COOH-THC in Δ9-COOH-THC Confirmation Samples

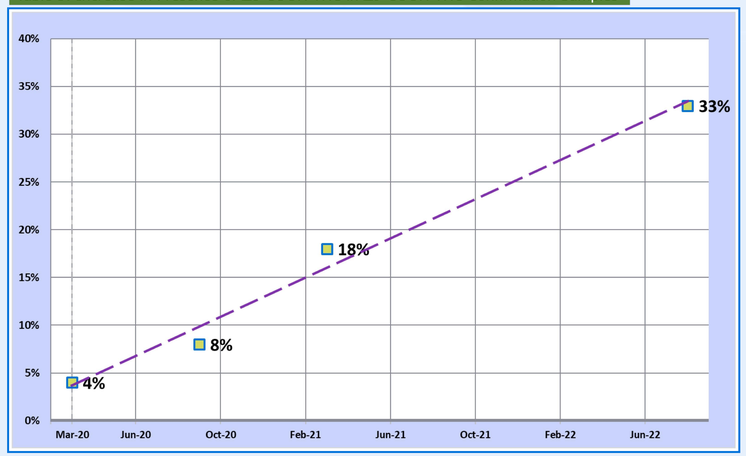


Table 4: Regulated Urine Specimen Δ9-COOH-THC Confirmation Rate
(Nonconfirming samples due to Δ8-COOH-THC interference)

Month	Confirmation Rate
Aug 2020	95%
Sept 2020	96%
Oct 2020	95%
Nov 2020	93%
Dec 2020	92%
Jan 2021	93%
Feb 2021	92%
Mar 2021	90%
Apr 2021	87%
May 2021	86%
Jun 2021	85%
Jul 2021	82%
Aug 2021	84%
Sept 2021	82%
Oct 2021	80%
Nov 2021	82%
Dec 2021	82%
Jan 2022	81%
Feb 2022	76%
Mar 2022	77%
Apr 2022	78%
May 2022	78%
Jun 2022	78%
Jul 2022	79%

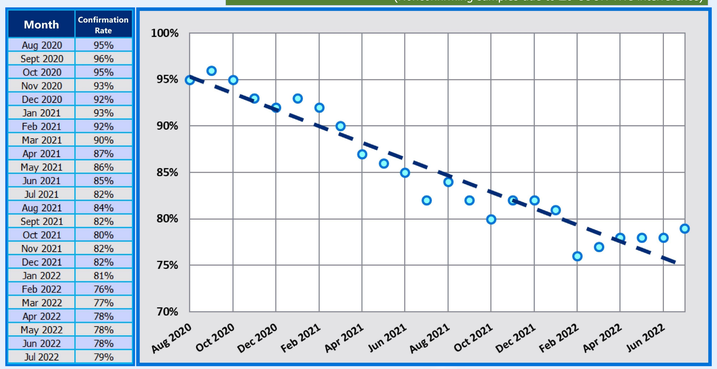
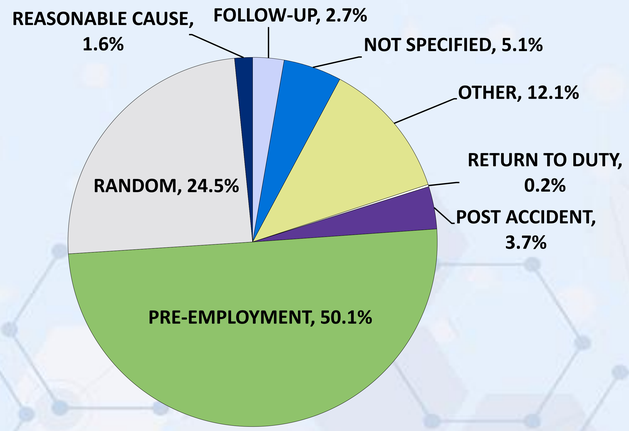


Figure E. Reasons for urine drug test for specimens with the observed presence of Δ8-COOH-THC (one week of data)



Periodic manual re-review of weekly LC-MS/MS confirmation data for regulated and non-regulated urine samples was performed to assess the rising trend of Δ9-COOH-THC positive specimens that also contain Δ8-COOH-THC. One week of data was categorized by reason for test, revealing that roughly half of the samples displaying Δ8-COOH-THC chromatography peaks were pre-employment urine drug tests.

CONCLUSION

Data trends indicate the increasing prevalence of Δ8-Carboxy-THC positive specimens going unreported. The federal drug testing program specifically identifies Δ9-Carboxy-THC as the reportable metabolite for marijuana use, and Δ8-THC products are specifically promoted as the "safe alternative" that is legal and/or can't be detected. Concentrations of Δ8-Carboxy-THC in the urine are often remarkably high, frequently into the hundreds of ng/mL and greater. The occurrence of Δ8-THC metabolite in regulated urine drug test samples indicates the possibility that individuals in safety-sensitive occupations may be operating while under the influence of THC-derivatives, all the while passing federal drug testing requirements. With the emergence of easily obtainable Δ8-THC products, employer drug testing programs should consider the inclusion of Δ8-Carboxy-THC in addition to Δ9-Carboxy-THC, as more than 20% of immunoassay results are negative with this omission.

REFERENCES

AB Sciex Turbo V™ Ion Source Operator Guide, 2012.
Adams R., Cain C.K., McPhee W.D., Wearn R.B. (1941). Structure of cannabidiol. XII. Isomerization to tetrahydrocannabinols. *Journal of the American Chemical Society, 63*(8), 2209–2213. https://doi: 10.1021/ja01853a052.
Cayman Chemical Reference Materials
Cerilliant Analytical Reference Materials
Mulé, S.J., Casella, G.A. (1988). Confirmation of Marijuana, Cocaine, Morphine, Codeine, Amphetamine, Methamphetamine, Phencyclidine by GC/MS in Urine Following Immunoassay Screening. *Journal of Analytical Toxicology 12*(2). 102-107. https://doi.org/10.1093/jat/12.2.102
NLCP Manual for Urine Laboratories, October 2017.
Paul, B.D., Mell, L.D., Mitchell, J.M., McKinley, R.M. (1987). Detection and Quantitation of Urinary 11-nor-delta 9-tetrahydrocannabinol-9-carboxylic acid, a Metabolite of Tetrahydrocannabinol, by Capillary Gas Chromatography and Electron Impact Mass Fragmentography. *Journal of Analytical Toxicology, 11*(1), 1-5. https://doi.org/10.1093/jat/11.1.1
Reber, J.D., Kanschner, E.L., Seither, J.Z., Knittel, J.L., Dozier, K.V., Walterscheid, J.P. (2022). An Enhanced LC–MS-MS Technique for Distinguishing Δ⁹- and Δ⁸-Tetrahydrocannabinol Isomers in Blood and Urine Specimens. *Journal of Analytical Toxicology, 46*(4), 343-349. https://doi.org/10.1093/jat/bkac007
Sciex, 6500 and 6500+ Series of Instruments System User Guide, 2015.
Snyder, L.R. (1972). A Rapid Approach to Selecting the Best Experimental Conditions for High-Speed Liquid Column Chromatography. Part I—Estimating Initial Sample Resolution and the Final Resolution Required by a Given Problem. *Journal of Chromatographic Science, 10*(4), 200-212. https://doi.org/10.1093/chromsci/10.4.200

DISCLOSURE

No relevant financial or nonfinancial relationships to disclose.