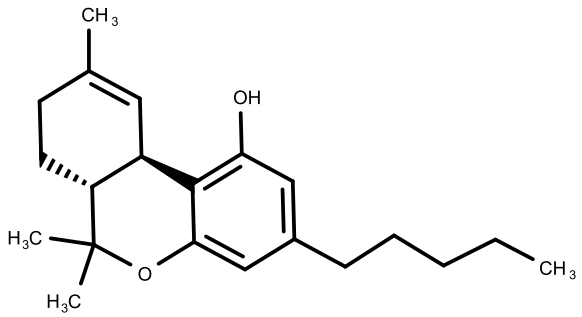
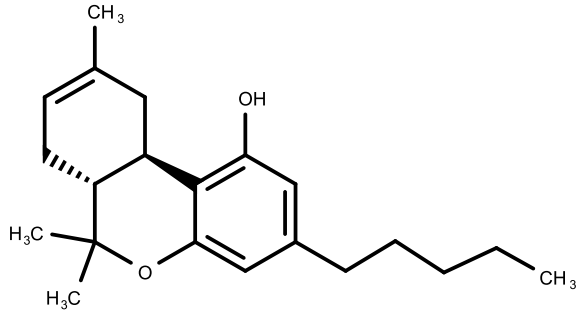
2020

Effects of Δ8-THCA on Initial and Confirmatory Testing for Cannabinoids in Urine

Delta-8-tetrahydrocannabinol (Δ8-THC) is a compound found in low abundance in cannabis plant material. It is formed as a degradation product of delta-9-tetrahydrocannabinol (Δ9-THC) in the cannabis plant or by Lewis acid catalyzed conversion of cannabidiol (CBD) or Δ9-THC.[1] It has been studied as a potential anti-cancer agent[2] and as an anti-emetic in pediatric oncology.[3] In recent years, interest has grown in producing and selling Δ8-THC as medicinal or recreational cannabis products, resulting in the proliferation of Δ8-THC vape liquids, tinctures, and edibles. As the use of Δ8-THC increases, some drug testing laboratories have begun to see more cannabinoid samples test positive for this compound or its metabolites.



Δ9-THC

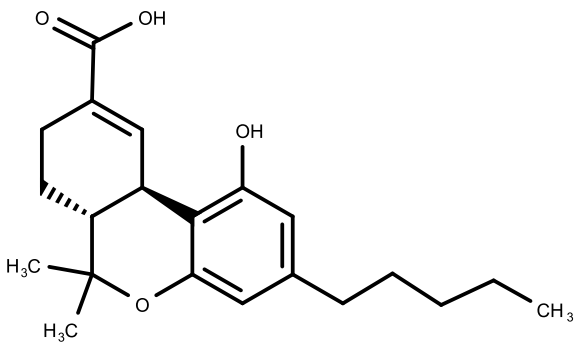


Δ8-THC

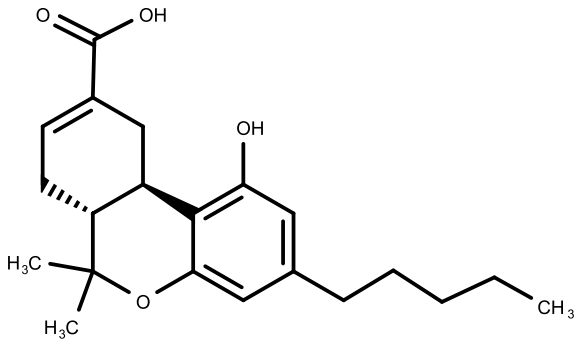
Effects of Δ8-THCA on Initial and Confirmatory Testing for Cannabinoids in Urine

The expected urinary metabolite of Δ8-THC is 9-carboxy-11-nor-delta-8-tetrahydrocanabinol (Δ8-THCA). Because of structural similarities, the presence of Δ8-THCA in a urine specimen might interfere with testing for Δ9-THCA. To assess the effect of Δ8-THCA on drug tests for Δ9-THCA, we prepared a special proficiency testing (PT) set consisting of samples spiked with combinations of Δ9-THCA and Δ8-THCA. We investigated two issues concerning Δ8-THCA:

1. How much does Δ8-THCA cross-react with the immunoassays used by U.S. Department of Health and Human Services (HHS)-certified laboratories?
2. Are laboratories able to confirm and quantify Δ9-THCA successfully in the presence of Δ8-THCA using their current confirmatory methods?



Δ9-THCA



Δ8-THCA

Table 1 shows the composition of the 11 samples in the special PT set. Each sample contained a total of 100 ng/mL THCA beginning with Sample 1, which was spiked with 100 ng/mL Δ9-THCA. In each successive sample, the Δ9-THCA concentration decreased by 10 ng/mL, whereas the Δ8-THCA concentration increased by 10 ng/mL. The final sample contained a concentration of 100 ng/mL Δ8-THCA.

Laboratories were directed to test the samples using their current initial and confirmatory test procedures for THCA. Laboratories were also instructed to submit their immunoassay data for review.

Table 1. Composition of Urine PT Samples

Sample Number	Δ8-THCA (ng/mL)	Δ9-THCA (ng/mL)
1	0	100
2	10	90
3	20	80
4	30	70
5	40	60
6	50	50
7	60	40
8	70	30
9	80	20
10	90	10
11	100	0

Initial Test Results

To evaluate the cross-reactivity of Δ8-THCA with the immunoassay reagents used by the laboratories, we compared the results of Sample 1 (100 ng/mL Δ9-THCA) and Sample 11 (100 ng/mL Δ8-THCA). The results are shown in **Table 2**. Note that results are reported in three different ways:

1. Semi-quantitative: Results are reported as concentrations of cannabinoids based on calibration with 50 ng/mL Δ9-THCA as 50. Results from 100 ng/mL samples will be near 100.
2. Normalized to 100%: Results are reported as percentages of cannabinoids based on calibration with 50 ng/mL Δ9-THCA as 100. Results from 100 ng/mL samples will be near 200.
3. Normalized to 1.000: Results are reported as ratios of cannabinoids based on calibration with 50 ng/mL Δ9-THCA as 1.000. Results from 100 ng/mL samples will be near 2.000.

The four immunoassays used by the laboratories were ThermoFisher DRI, Siemens EMIT 5B3 THC, Roche KIMS, and Siemens EMIT II Plus.

Immunoassay results for Δ9-THCA and Δ8-THCA were very similar for all laboratories and all reagents, with an average cross-reactivity of 99%.

Table 2. Initial Test Results for Δ9-THCA (100 ng/mL) vs. Δ8-THCA (100 ng/mL)

Initial test cutoff concentration				
Lab ID	**Reagent**	**100 ng/mL delta-9 THCCOOH Response**	**100 ng/mL delta-8 THCCOOH Response**	**Cross-Reactivity (%)**
1	ThermoFisher, DRI	217	213	98
2	ThermoFisher, DRI	108	107	99
3	Siemens, EMIT 5B3 THC	211	201	95
4	ThermoFisher, DRI	1.884	1.912	101
5	ThermoFisher, DRI	1.819	1.812	100
6	ThermoFisher, DRI	87	85	98
7	ThermoFisher, DRI	100	95	95
8	Roche, KIMS	155	143	92
9	ThermoFisher, DRI	142	125	88
10	Siemens, EMIT II Plus	257	247	96
11	ThermoFisher, DRI	136	131	96
12	Siemens, EMIT II Plus	107.64	102.09	95
13	Siemens, EMIT II Plus	77	78	101
14	ThermoFisher, DRI	96	97	101
15	ThermoFisher, DRI	1.848	1.836	99
16	ThermoFisher, DRI	94	94	100
17	ThermoFisher, DRI	189	185	98
18	Siemens, EMIT II Plus	114	107	94
19	Siemens, EMIT II Plus	151	157	104
20	Siemens, EMIT II Plus	112	125	112
21	ThermoFisher, DRI	60	62	103
22	ThermoFisher, DRI	137.2	137.2	100
23	ThermoFisher, DRI	99	95	96
24	ThermoFisher, DRI	204	208	102
			Average	99

Confirmatory Test Results

Laboratories were instructed to perform THCA confirmatory testing on all 11 special PT samples using their current analytical procedures. Group mean Δ9-THCA test results are shown in **Table 3**. Mean test results agreed well with target concentrations.

Table 3. Δ9-THCA Mean Test Results

Initial test cutoff concentration			
Sample Number	Δ8-THCA (ng/mL)	Δ9-THCA (ng/mL)	Δ9-THCA Mean (ng/mL)
1	0	100	104.2
2	10	90	93.5
3	20	80	83.0
4	30	70	73.1
5	40	60	61.6
6	50	50	52.3
7	60	40	41.9
8	70	30	30.4
9	80	20	20.5
10	90	10	10.2
11	100	0	No Data

Table 4 shows the confirmatory test data that exhibited interference. Of the 23 laboratories, 20 were able to detect and quantify Δ9-THCA successfully in the presence of Δ8-THCA in all samples. Three laboratories reported varying difficulties with the PT set:

- Lab 10 was not able to confirm Δ9-THCA in any of the samples that also contained Δ8-THCA (Samples 2–10) because of chromatographic interference and mass ratio failure.
- Lab 17 was unable to confirm Δ9-THCA when the concentration of Δ8-THCA reached 70 ng/mL and Δ9-THCA decreased to 30 ng/mL (Samples 8–10) because of chromatographic interference and mass ratio failures.
- Lab 18 was unable to confirm Δ9-THCA when the concentration of Δ8-THCA reached 20 ng/mL and Δ9-THCA decreased to 80 ng/mL (Samples 3–10) because of chromatographic interference.

No laboratory reported Sample 11 (100 ng/mL Δ8-THCA) as positive for Δ9-THCA.

Table 4. Interference Observed in Confirmatory Testing

Lab ID	Derivatizing Agent	Interference by Sample
2	None	No Interference
3	BSTFA	No Interference
4	BSTFA	No Interference
5	BSTFA	No Interference
6	BSTFA	No Interference
7	MTBSTFA	No Interference
8	TBDMS	No Interference
9	BSTFA	No Interference
10	C3H7I	Samples 2–10
11	MTBSTFA	No Interference
12	BSTFA	No Interference
13	BSTFA	No Interference
14	MTBSTFA	No Interference
15	BSTFA	No Interference
16	MTBSTFA	No Interference
17	CH3I	Samples 8–10
18	BSTFA	Samples 3–10
19	MSTFA	No Interference
20	MTBSTFA	No Interference
21	MTBSTFA	No Interference
22	MTBSTFA	No Interference
23	MTBSTFA	No Interference
24	BSTFA	No Interference

Confirmatory Test Results from Selected Laboratories

Lab 10 used propyl derivatization in their confirmatory method for THCA. The laboratory changed from the use of pentafluoropropionic acid and pentafluoropropyl alcohol (PFPA/PFPOH) because of issues with conversion of CBD-based compounds to THC-based compounds. Under this laboratory's gas chromatograph conditions, Δ9-THCA and Δ8-THCA had the same retention time (3.5 minutes) and eluted as a single peak. However, the fragmentation patterns of the two (dipropyl) derivatized molecules are very different. The derivatized Δ9-THCA is highly fragmented with a base peak of 341 and a relatively low-abundance (20%) molecular ion of 428 (**Figure 1**). However, the derivatized Δ8-THCA shows the molecular ion (428) as the base peak and is fragmented very little, with a 341 peak of only 4%. This difference in fragmentation is consistent with what is known about the relative stabilities of Δ9-THC and Δ8-THC. Although Δ9-THC is the predominant compound formed biosynthetically in the cannabis plant, Δ8-THC is more thermodynamically stable. Apparently, this difference in structural stability is also the case with carboxy metabolites.

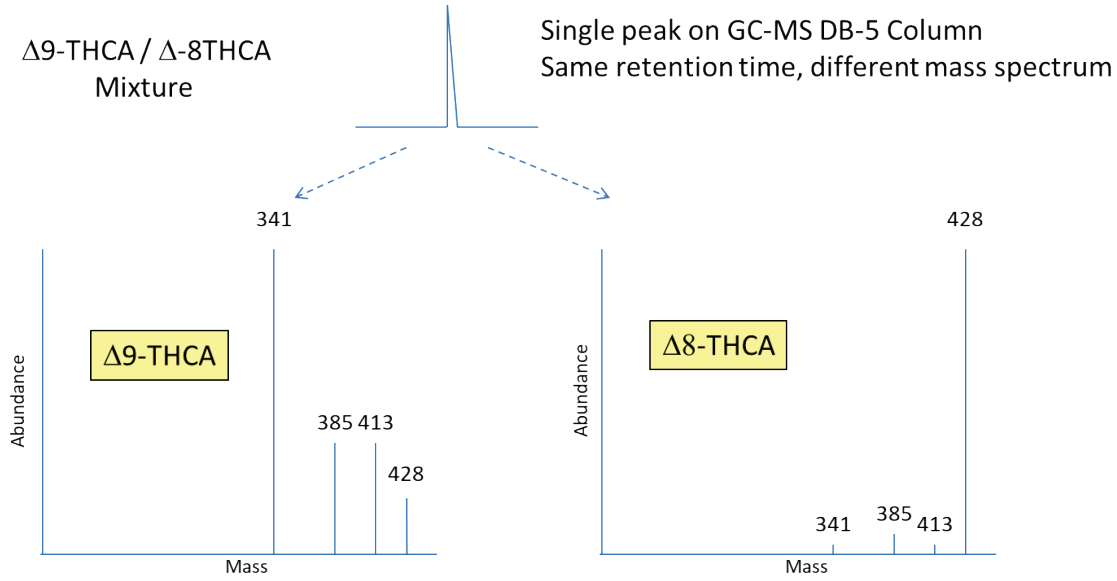


Figure 1. Selected Ion Mass Spectra of Δ9-THCA and Δ8-THCA (Lab 10 dipropyl derivate)

Table 5. Δ9-THCA Mean Test Results

THCA Iodopropane Confirmation Method		
	Δ9-THCA	Δ8-THCA
Ion	Relative Abundance (%)	
428	20	100
341	100	4
385	41	8
413	40	5

Confirmatory Test Results from Selected Laboratories

Lab 17 uses a dimethyl derivative of THCA. Because of a long run time (retention time = 8.378 minutes), this laboratory was able to achieve some separation of Δ9-THCA and Δ8-THCA. However, when Δ8-THCA concentration reached 70 ng/mL (with Δ9-THCA at 30 ng/mL), the laboratory was not able to achieve acceptable chromatography for the 372-qualifier ion (i.e., the molecular ion; **Figure 2**). Like Lab 10, Lab 17 experienced interference because of the relatively large molecular ion from Δ8 THCA. Although the mass ratio was acceptable, the valley between the Δ9-THCA and Δ8-THCA peaks was greater than 10% of the Δ9-THCA peak height (**Figure 3**).

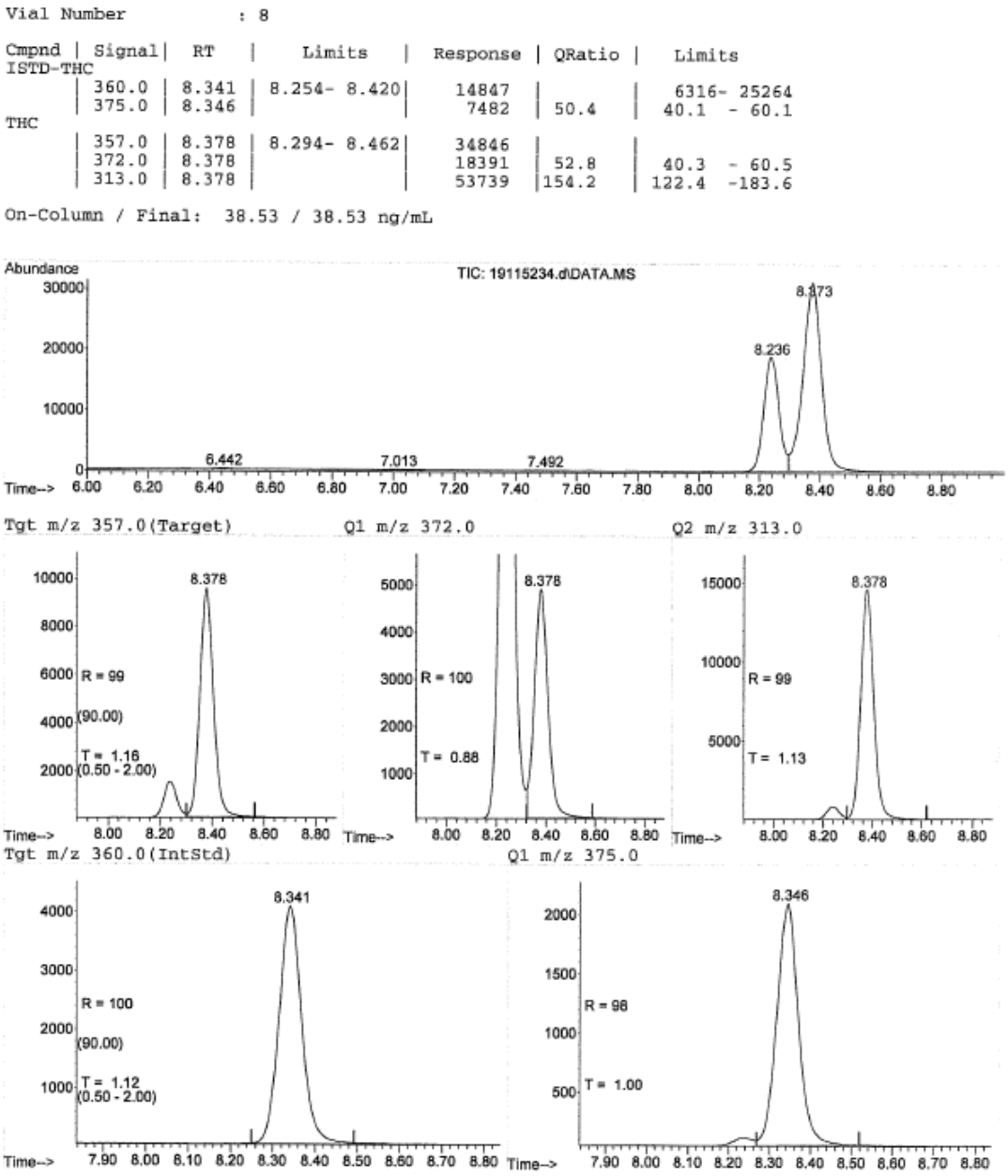


Figure 2. THCA Chromatogram (Lab 17 dimethyl derivate)

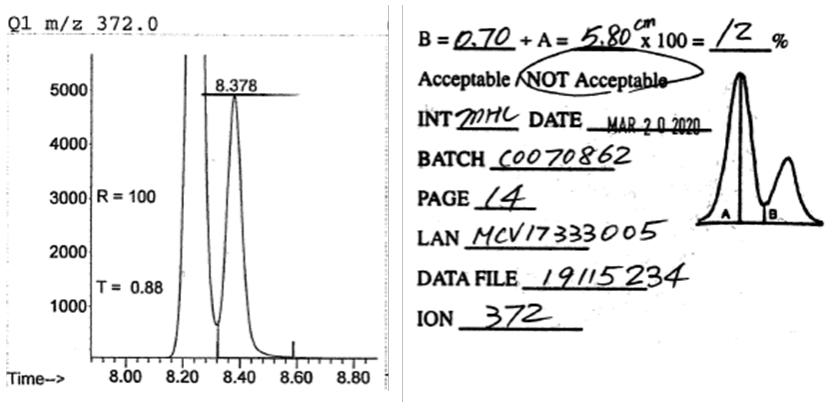
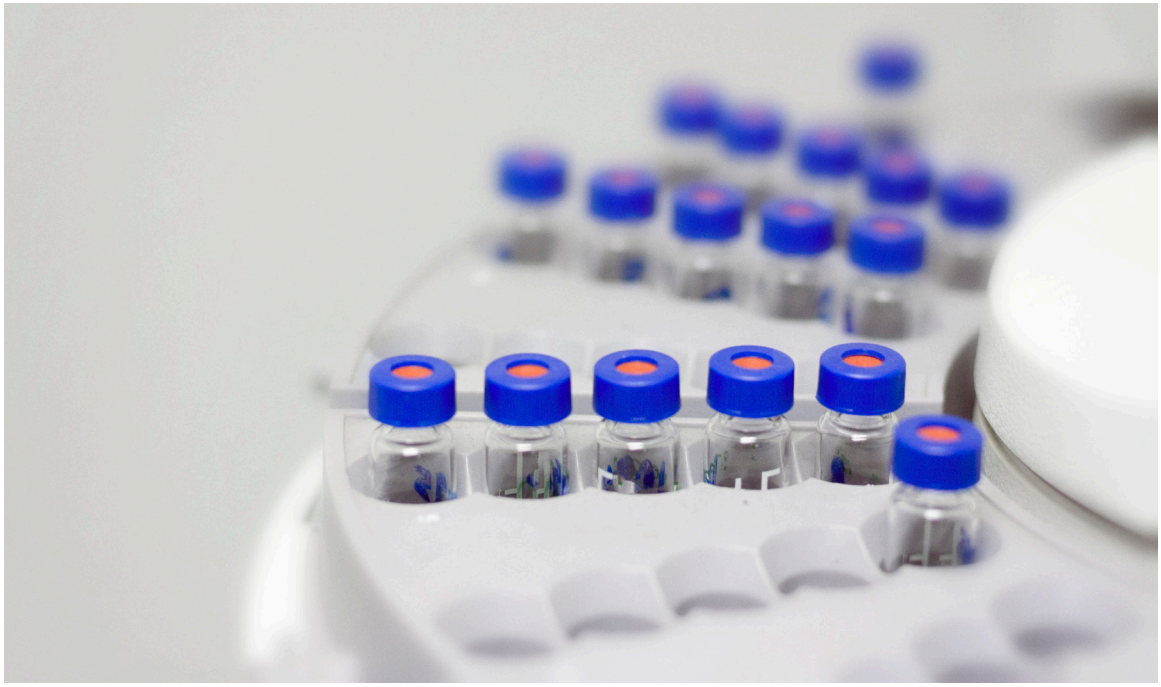


Figure 3. Peak Resolution for THCA (Lab 17 dimethyl derivate) 372 ion



Lab 18 uses a bis-TMS derivative of THCA. Because of a relatively short run time (retention time = 1.6 minutes), this laboratory was not able to achieve complete separation between Δ9-THCA and Δ8-THCA when the concentration of Δ8-THCA reached 20 ng/mL. In addition to chromatographic interference, the laboratory experienced mass ratio failures at *m/z* 488 (molecular ion) and *m/z* 473 (**Figure 4**).

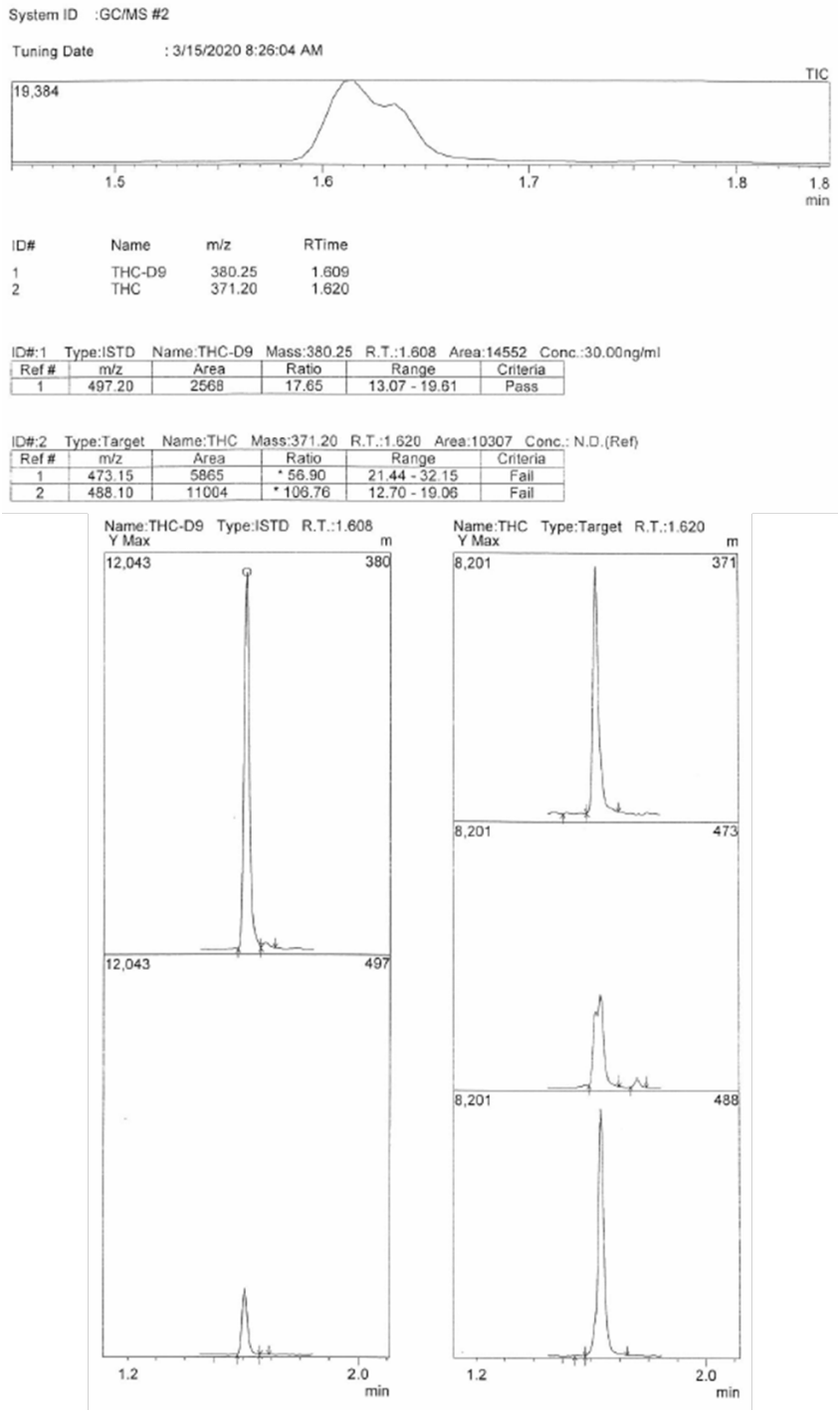


Figure 4. THCA Chromatogram (Lab 18 bis-TMS derivate)

Conclusion/Discussion

In answer to our first question about the cross-reactivity of Δ8-THCA, we learned that Δ8-THCA is highly cross-reactive with the immunoassay reagents used by HHS-certified laboratories. Therefore, specimens containing Δ8-THCA could give positive initial test results even with an absence of Δ9-THCA because of the high immunoreactivity of Δ8-THCA.

Our second question was whether the HHS-certified laboratories can successfully confirm and quantify Δ9-THCA in the presence of Δ8-THCA using their current confirmatory methods. We learned that the presence of Δ8-THCA can result in false negative results for Δ9-THCA because of chromatographic interference or mass ratio failures. However, there were no false positive Δ9-THCA reports. Previous reports in the literature indicate that laboratories using fast chromatography liquid chromatography–mass spectrometry procedures may be at risk of false positives for Δ9-THCA when Δ8-THCA is present in a specimen. However, we were not able to evaluate that issue with this PT set.

Based on our observations in this special PT set, we recommend that laboratories validate their cannabinoid confirmatory methods to ensure that they do not experience interference from the presence of Δ8-THCA.

References

1. 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.
2. 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.
3. Abrahamov, A., Abrahamov, A., & Mechoulam, R. (1995). An efficient new cannabinoid antiemetic in pediatric oncology. *Life sciences, 56*(23-24), 2097–2102.

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 he also serves as an NLCP inspector. In addition to his work with the NLCP, he participates in CFS Research and Development projects and manages RTI's Oral Fluid Proficiency Testing Program. Before joining RTI, Mr. Hart worked in forensic drug testing laboratories for the U.S. military and in the private sector, including work at an HHS-certified laboratory as a laboratory manager and expert witness.