CANNABINOIDS IN ORAL FLUID: LIMITING POTENTIAL SOURCES OF CANNABIDIOL CONVERSION TO Δ9 - AND Δ8 - TETRAHYDROCANNABINOL

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Abstract: In late 2019, the National Laboratory Certification Program (NLCP) published an article reporting on the potential analytical conversion of 7-carboxy-cannabidiol (CBD-COOH) to 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine samples. The same conversion is possible in oral fluid with the parent analyte cannabidiol (CBD) converting to Δ9-tetrahydrocannabinol (Δ9-THC) and Δ8-tetrahydrocannabinol (Δ8-THC) under strong acidic conditions. With the recent rise in states legalizing the use of THC and the availability of products purportedly containing only CBD, unless the analytical *in vitro* conversions are controlled, the detection of Δ9-THC or Δ8-THC in oral fluid may not clarify whether the donor was using a CBD product, licit or illicit THC product. Authentic oral fluid samples submitted for cannabinoid analysis were subjected to multiple sample preparation procedures and extraction methods to determine the conditions that allow CBD to convert to THC. CBD single analyte controls prepared from a certified THC-free source were added to the batch to monitor the rate of conversion. Samples were prepared using a base hydrolysis, solid phase extraction, derivatization, and analysis by liquid chromatography with tandem mass spectrometry (LC–MS/MS). The base hydrolysis and derivatization were tested independently and did not contribute to the conversion rate. Adjusting the pH of the sample preparation and extraction from pH 2.0 to pH 5.0 changed the conversion rate from 5% to 1%. A pH of 6.0 was not strong enough to extract the cannabinoids efficiently. Removing the acid component of the preparation and extraction procedure eliminated the conversion to THC; however, this did reduce the analyte recovery depending on which extraction column was used. Processing time also contributed to the conversion rate. With smaller trial runs, conversion was not always seen but with larger validation batches low level conversion of 1–2% was observed. A fully validated LC–MS/MS method utilizing solid-phase extraction was developed for CBD, Δ9-THC, Δ8-THC, and cannabinol (CBN). The method specifically targets those analytes found in oral fluid after CBD administration and those that are seen during *in vitro* CBD conversion.

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CHAPTER I

INTRODUCTION

In late 2019, the National Laboratory Certification Program (NLCP) published an article reporting on the potential analytical conversion of 7-carboxy-cannabidiol (CBD-COOH) to 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine samples. (1) In 2012, Andrews and Paterson illustrated the potential cannabidiol (CBD) conversion to Δ9-tetrahydrocannabinol (THC) when an acidic derivative was used, and samples were analyzed by gas chromatography–mass spectrometry (GC–MS) (2). The team noticed when samples were derivatized by trifuoroacetic anhydride and hexafluoroisopropanol (TFAA-HFIP), CBD and THC both gave the same structure and peak at the same retention time. They also noticed two additional peaks that corresponded to Δ8-THC and an intermediate peak. The work done by Andrews and Paterson was on spiked methanolic drug standards and did not correspond to a biological matrix (i.e., urine or oral fluid).

Work done by the National Laboratory Certification Program (NLCP) published in 2019 involved the organization sending blind 11- nor-9-carboxy-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) / 7-carboxy-cannabidiol (CBD-COOH) urine proficiency samples to two selected laboratories and requesting analysis be done using either pentafluoropropionic anhydride and pentafluoropropanol (PFPA-PFPOH) or pentafluoropropionic anhydride and hexafluoroisopropanol (PFPA-HFIP) derivatives and analysis by GC-MS. Both derivatives are considered strong acid derivatives. Both laboratories reported values that did not correlate with

expected results. The laboratory using PFPA-PFPOH derivative reported a 4-12% conversion of CBD-COOH to THC-COOH and the second lab using PFPA-HFIP derivative reported a 136-163% conversion of CBD-COOH to THC-COOH (1, 3). The same conversion is possible in oral fluid with the parent analyte cannabidiol (CBD) converting to Δ9-tetrahydrocannabinol (Δ9-THC) and Δ8-tetrahydrocannabinol (Δ8-THC) under strong acidic conditions.

In 2020 Golombek et. al. published a review of both *in vitro* and *in vivo* CBD conversion studies. It was the team's findings that studies showed CBD converted to THC under acidic conditions, but these conversions did not occur *in vivo* and were not found in any animal or human studies. This finding correlates to the fact that the psychotropic effects of THC were not felt by the subjects in the studies (4). To date, THC is still considered a schedule 1 controlled substance. The 2018 Farm bill has removed CBD from the Controlled Substances Act, when CBD is derived from the Hemp plant and contains less than 0.3% Δ9-THC (5). With the recent rise in states legalizing the use of marijuana and a variety of cannabis products containing only CBD (<0.3% THC), there is a risk for the toxicologist to incorrectly identify whether the donor was using CBD or THC. Because many cannabinoid products on the market contain a mix of both CBD and THC, there is also a potential for over-or under-reporting of sample concentrations. Internet searches (as shown by Golombek, et. al.) for CBD have risen from less than 5% in 2013 to a 100% increase in 2019. The interest in CBD is due to the non-psychoactive properties of CBD and the homeopathic qualities (4). Users of CBD have reported anti-inflammatory, anxiolytic, analgesic effects, and positive effects on appetite. That said, research is ongoing and nothing appears to have been proven definitively to this point. The research team of Vazquez, et. al. showed quite the opposite effect when comparing drug-drug interaction of CBD and common opioids used in pain management. CBD inhibits CYP450 isoenzymes and by doing so, morphine and oxycodone potencies are reduced, at the same time concurrent use of CBD with methadone would cause methadone levels to rise, causing an increase in potency. The author states "cannabinoids inhibit

the activity of these isoenzymes and make normal metabolizers resemble poor metabolizers" (6). More studies are needed to confirm these findings, but the nonscientific public consensus is CBD is a safe and helpful supplement to take.

In most laboratory settings, samples are first processed through screening kits. Immunoassays on the market do not detect CBD in the cannabinoid screening profiles except at very high concentrations. The Immunalysis STHC ELISA kit states a CBD concentration of 10,000 ng/mL is needed to elicit a positive screen similar to Δ9-THC at 4 ng/mL (7). It is thought the risk for false positives created by high levels of CBD would be very low, and only the true positives for THC would move to confirmatory testing. However, a case where both CBD and THC are present could create an over- or under-reporting situation for both analytes.

In some cases, testing laboratories include steps in their protocols to confirm all samples regardless of initial screening results. Though a rare occurrence, this presents a potential for discrepant results, depending on the sample preparation for cannabinoid analysis. Targeted research projects where screening results are not of concern could also be a potential risk. The rise in liquid chromatography with tandem mass spectrometry LC-MS/MS screening could also create false results, as the samples must go through a cleanup preparation step before being analyzed by LC-MS/MS. This is the same process used for confirmation, and the same source for CBD to THC conversion. In this case the screen would match the confirmation. The objective of this work was to evaluate sources contributing to CBD converting to THC in oral fluid analysis through comparing data collected by several different sample preparation and extraction techniques, then determine if better extraction procedures can eliminate the potential for conversion. This will provide cannabinoid results that more accurately reflect true values.

CHAPTER II

REVIEW OF LITERATURE

Section 1: Literature Review

In addition to the studies referenced earlier, the NLCP have expanded their study of CBD and CBD metabolites as recently as May 2021 and are studying the conversion rate of 7-carboxy-cannabidiol (CBD-COOH) to 11-nor-9-carboxy-tetrahydrocannbinol (THC-COOH) (8). In this study proficiency samples containing a combination of CBD-COOH and/or THC-COOH were sent to 25 testing laboratories for analysis by both screening and confirmation techniques. The NLCP found in all cases the immunoassay screening results for CBD-COOH were negative. For confirmation results when laboratories used nonacidic derivatizing methods, conversion was either not seen or was kept below 0.42%. When the NLCP requested acid derivatization for confirmation analysis the conversion rates for CBD-COOH increased to between 4.7% – 12.8%. Also, when samples were exposed to acidic conditions for extended periods of time, as is the case with overnight sample preparation, conversion rates increased to 31% (8).

In 2020 Dybowski et. al. reported findings for CBD to convert to Δ9-THC during protein precipitation of whole blood during sample preparation when strong acids were used. Conversions rates seen were at most 20%. Dybowski also noted that exposure time played a role in higher conversion rates when a higher heat source was not available to enhance the reaction (9).

Section 2: Legal Fallout for False Δ9-THC Reporting

The legal ramifications for false THC reporting fall into two categories, those for the person under suspicion of use and the testing laboratory. For the individual this could be a workplace drug test in which case they could face loss of a job or opportunity for employment. The individual could be fighting a custody rights claim or an insurance claim. They could be denied life insurance or face increased rates for car insurance. They could be on parole and a positive THC confirmation test could send them back to jail. While it is true states that have legalized cannabis or at the least decriminalized it both the state and federal law enforcement have taken a relaxed approach to enforcement (10), it does remain that employers generally have the right to maintain a drug free workplace and issue random employee drug tests.

The testing laboratory must put into place safeguards to monitor for the potential conversion of CBD. They should have a validated method that shows no amount of CBD can be converted to THC during the sample processing and analysis. More proficiency programs have started to add CBD to their testing profiles. This is a good way to monitor externally that methods are performing optimally. If labs fail their proficiency testing, the accreditation body could revoke their ability to perform, and report results for the cannabinoid assay. Toxicologists and chemists could face a line of questioning during trial about the performance of their assay and may need to provide proof the method does not show any conversion of CBD to THC. If the laboratories are unable to do this, they may lose their reputation in the criminal justice system or accreditation by a governing body.

CHAPTER III

METHODOLOGY

Section 1: Authentic Specimens and Original Analysis

Quantisal™ collected oral fluid samples received for routine cannabinoid analysis were originally analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) on a cannabinoid panel including Δ9-THC, Δ8-THC, THC-COOH, CBD, CBD-COOH, and cannabinol (CBN). Parent analytes THC and CBD were expected to be present in the samples. Metabolites had the potential to be present at much lower concentrations depending on dosage and time of collection. Sample preparation for oral fluid included a base hydrolysis to cleave any glucuronide bound THC-COOH. The base hydrolysis was neutralized by adding 500 µL of acetic acid. It was believed adding a strong acid may increase the potential for CBD and CBD-COOH to convert to THC and THC-COOH. The lowered cut off values for THC of 1 ng/mL and THC-COOH at 20 pg/mL would be problematic even for a low percentage conversion. Samples were extracted by solid phase extraction using an acid neutral procedure. Samples were eluted using hexane with 2% acetic acid, evaporated, and derivatized using 2-picolamine (2-PA), triphenylphosphine (TPP) and 2,2’-dipyridyl disulfide (DPDS). This was a basic derivative used to enhance only the THC-COOH signal; other cannabinoids were not affected. Samples were analyzed by LC–MS/MS in positive electrospray ionization (+ESI) mode with the mobile phase gradient being slightly acidic. The original method outlined was part of a previously validated and published cannabinoids method (11). This study showed that when screened by ELISA, these oral fluid samples were

negative below 4 ng/mL. Samples analyzed by LC–MS/MS confirmation were positive for both Δ9-THC and Δ8-THC and for CBD. CBD's cross reactivity with the STHC ELISA kit is very low with 10,000 ng/mL needed to cause a positive absorbance and was not believed to be contributing to the positivity of a screened sample.

The original method described above caused around 5% of CBD to be converted *in vitro* to THC during the sample preparation process. No CBD-COOH or THC-COOH were found in any of the oral fluid samples; therefore, base hydrolysis and derivatization will not be included in future method development aimed at eliminating CBD conversion.

Section 2: Materials - Supplies and Reagents

Quantisal™ buffer and negative synthetic oral fluid manufactured by Immunalysis Corporation (Pomona, CA) were used for calibrator preparation. Calibrators mimic the 3-parts Quantisal™ buffer and 1-part oral fluid collection protocol of authentic samples. The concentrations of the prepared calibrators and controls are adjusted to one fourth the concentration and reported as neat concentration values. The Quantisal™ collection device collects approximately 1 mL neat oral fluid (±10%) on a cotton pad, which is then placed in a tube with 3 mL of transportation buffer. When analyzed by a lab, concentration values must be represented as neat oral fluid values. Deuterated d3-THC and d3-CBD (internal standards), and drug standards Δ9-THC, Δ8-THC, CBD, and CBN were all purchased from Millipore (Round Rock, TX). Solid phase extraction columns, Trace-N 3 mL 15 mg and Cerex Polycrom THC 3mL 35 mg, were purchased from Tecan (formerly SPEWare, Baldwin Park, CA). Solvents and chemicals were all purchased from Fisher Scientific and were HPLC grade or better. Calibration curves were prepared in 1 mL Quantisal buffer and negative synthetic oral fluid keeping with the 3-parts buffer + 1-part oral fluid dilution. Δ9-THC, Δ8-THC, CBD, and CBN were prepared at levels of 1, 2, 4, 10, 50, 100 ng/mL. Because of the potential for CBD conversion to Δ9-THC and Δ8-THC, a separate CBD

external control from a different source material was used as a single analyte control. CBD at 100 ng/mL was prepared from a certified THC-free standard and run with each batch of samples.

Section 3: Sample Preparation

Sample preparation was believed to be the source of the conversion. By looking at each step in the extraction process as a variable the goal was to isolate the problematic step and find a better solution.

Base Hydrolysis. Though ultimately not used for this method development, the hydrolysis component was tested as a source for CBD conversion. Two curves were prepared from 5 ng/mL to 100 ng/mL. One curve was hydrolyzed using a 1N sodium hydroxide solution and neutralized with 0.5 mL glacial acetic acid. The second curve was not hydrolyzed. When isolated, the addition of glacial acetic acid did not affect the conversion of CBD to THC and did not contribute to the overall percentage of conversion in any of the original samples tested. This could be because the samples were only exposed to the acid for a short amount of time and the neutralization with the glacial acetic acid did not leave the resulting pH to be acidic enough to contribute to conversion.

Solid-Phase-Extraction Columns (SPE). Two SPE columns were tested for suitability, Trace-N and Cerex Polycrom THC both by Tecan (Baldwin Park, CA). See Table 1 for a preparation outline.

Method	Sample pre treatment	Column conditioning	Column washing	Eluant
Trace-N	Base hydrolysis 500 µL glacial acetic acid	500 µL methanol 100 µL 0.1M acetic acid	1 mL 80:20 acetic acid: water 1 mL 60:40 water: methanol	1 mL 98:2 Hexane: glacial acetic acid
Trace-N	Base hydrolysis 500 µL glacial acetic acid	500 µL methanol 100 µL pH 4.0 acetic acid	1 mL 60:40 water: methanol	1 mL 98:2 Hexane: glacial acetic acid
Cerex Polycrom THC	2 mL DI water	none	1 mL 90:10:1 water: acetonitrile: ammonium hydroxide	2 mL ethyl acetate

				2 mL 88:10:2 Hexane: ethyl acetate: acetic acid

Table 1. Sample preparation outline for Trace-N and Cerex Polycrom THC columns.

Trace-N sorbent is described as a hydrophobic polymer with weak anion exchange. Cerex Polycrom THC sorbent is a polymeric reverse phase with anion exchange characteristics. Trace-N columns require samples to be loaded, washed, and eluted under acidic conditions to maintain high extraction efficiency. A 0.1M acetic acid solution (pH 2.0) was used for the column conditioning and an 80% glacial acetic acid solution was used to wash the columns after sample loading. These two steps combined led to a 5% conversion of CBD to Δ9- THC and Δ8-THC. When the pH of the column conditioning was changed to 4.0 and the acid wash step was removed the CBD to THC conversion rate reduced to 1% and 0.5% for Δ9-THC and Δ8-THC, respectively. The Cerex Polycrom THC columns did not require an acid component for loading and washing but did have a similar hexane with 2% acetic acid eluant. The eluant and exposure during evaporation did not contribute to either of the conversion rates. When used, the Cerex Polycrom THC showed no conversion of CBD to Δ9-THC or Δ8-THC. The final validated extraction procedure for the Cerex columns was to add 2 mL DI water to 1 mL of Quantisal™ collected oral fluid sample and load directly on an unconditioned Cerex column. Columns were washed with 1 mL 90:10:1 water: acetonitrile: ammonium hydroxide solution then dried for 15 minutes. Columns were eluted first with 2 mL ethyl acetate, dried for 10 minutes, and eluted a second time with 2 mL 88:10:2 hexane: ethyl acetate: acetic acid solution. The collected eluant was dried completely under nitrogen at 30 psi and reconstituted in 50:50 water: acetonitrile solution. Percent conversions of CBD to Δ9-THC and Δ8-THC for each column tested are presented in Table 2.

	Average % Conversion of CBD	
Method	**Δ9-THC**	**Δ8-THC**
Trace-N	5	5
Trace-N	1.1	0.5

Cerex Polycrom THC	0	0

Table 2. Average percent conversion of CBD to Δ9-THC and Δ8-THC for each column.

The results show on average 5% of CBD was converted to Δ9-THC and Δ8-THC when using the original method. The percent conversion was reduced to 1.1% and 0.5% for Δ9-THC and Δ8-THC with the reduction of acid during the extraction process and was completely eliminated when using the Cerex column method.

Batch size and Extraction time. During the method development process, several small trial runs isolating single variables at times showed no conversion. When these changes were implemented to a larger batch, increased rates of conversion were seen. It was thought that CBD might experience longer exposure times to acid with larger batches that involved more sample processing time. This may make it difficult for laboratories to properly validate a method if multiple analysts have slightly different extraction speeds. This was only seen when using Trace-N columns and not during the method development using Cerex Polycrom THC columns.

Section 4: LC-MS/MS Conditions

During method development no detectable conversion of CBD to THC was noticed when the LC-MS/MS conditions were isolated as a variable. To isolate the LC-MS/MS conditions a 5000 ng/mL CBD standard was prepared as a methanolic solution, evaporated, and reconstituted in 50:50 water: acetonitrile. The standard was injected directly and through an analytical column into the LC-MS/MS and analyzed for Δ9-THC and Δ8-THC. No conversion was seen; therefore, this portion of the method was not altered for any of the conditions tested.

Liquid Chromatography Conditions. A previously published and validated LC-MS/MS method was used as the basis for this study (11). Minor optimizations were made and CBD, CBN, and Δ8-THC was added. An Agilent Technologies 1200 series binary pump coupled to an Agilent 6430 tandem mass spectrometer operating in positive electrospray ionization (+ESI) mode were

used for analysis. 20 µL of sample was injected on to an Eclipse Plus C18 2.1x50mm column with 5mM ammonium formate mobile phase A (MPA) and 0.5% formic acid in acetonitrile solution as mobile phase B (MPB). The acidic nature of the mobile phase did not influence CBD conversion. This was tested against the single analyte CBD control standard at 5000 ng/mL. Column temperature was held at 60ºC. Time 0 MPB started at 70% and held for 2 minutes, at 4 minutes MPB was increased to 90%. Stop time was at 9 minutes with an 8-minute post run period to re-equilibrate the gradient and column before the next injection. Tandem Mass Spectrometer conditions. For all time segments the nebulizer gas temperature was held at 300ºC, gas flow held at 10 L/min, nebulizer pressure held at 50 psi, and positive capillary voltage held at 3500V. MRM mode scans for d3-Δ9-THC transition 318 > 196 using fragmentor voltage 150 and collision energy 20, d3-CBD transition 318 > 196 using fragmentor voltage 150 and collision energy 20, and Δ9-THC transitions 315 > 193 and 123 using fragmentor voltage 120 and collision energy 20 and 25, respectively. CBN transitions of 311 > 223 and 195 using fragmentor voltage 120 and collision energy 20 and 25, respectively. Transitions for CBD and Δ8-THC were the same as Δ9-THC. The analytes were chromatographically separated and identified based on retention time. CBD elutes early at 3.1 minutes and Δ9-THC will elute at 5.2 minutes with Δ8-THC at 5.5 minutes. Baseline separation was difficult to achieve between Δ9-THC and Δ8-THC.

CHAPTER IV

FINDINGS

Section 1: Method Validation

Method validation processes followed guidance in ASB standard 036, first edition 2019 Standard Practices for Method Validation in Forensic Toxicology (12).

Bias and Precision. Bias and precision samples were run concurrently, and the same samples were used to populate both validation criteria. Positive quality control samples for CBD, CBN, Δ9-THC, and Δ8-THC were 1, 2, and 20 ng/mL for low, medium, and high, respectively. Concentrations were listed as LOW, MED, HIGH. Bias of ±20% was considered acceptable for each of the three concentrations levels run in triplicate over five separate runs. Table 3 shows % bias for each analyte at each concentration, which met acceptance criteria within a 20% variance.

Level	CBD	CBN	Δ9-THC	Δ8-THC
Low	10.73	17.80	15.33	14.40
Medium	1.63	17.00	9.17	9.07
High	-4.11	-3.49	-1.13	0.50

Table 3. % Bias calculated over triplicate analysis for 5 runs

Precision samples were run at three levels in triplicate over five runs, where calculated % CV should be less than 20%. Table 4 summarizes the precision data, which was within 20% range for acceptance.

Level	CBD	CBN	Δ9-THC	Δ8-THC
Low	8.81	3.92	3.37	9.63
Medium	4.86	5.32	6.47	5.84
High	6.23	5.71	4.82	8.65

Table 4. Precision % CV calculated over triplicate analysis for 5 runs

Calibration. The calibration model fit was linear with the weighting calculated as 1/x, as concentration spanned greater than 1 order of magnitude. The standard deviation of the slope and y-intercepts were evaluated and were not outside ±3 standard deviations. Table 5 shows the standard deviation of the slope and y-intercepts for each analyte.

	CBD	CBN	Δ9-THC	Δ8-THC
Slope	0.030	0.029	0.044	0.049
y-intercept	0.061	0.231	0.033	0.096

Table 5. Standard deviation calculated for slope and y-intercept for 5 runs

Carry Over. To assess carry over a blank sample was analyzed after each of the highest calibrators and after the CBD single analyte external control. No peaks were found for any analyte or deuterated analog for each of the 5 runs.

Interference. Authentic human oral fluid from 10 different donors was analyzed with no internal standard to determine any interference derived from matrix background. No interference was seen for any of the donor samples. These commonly tested analytes were added to synthetic negative oral fluid at a concentration of 10,000 ng/mL; morphine, codeine, 6-acetylmorphine, oxycodone, oxymorphone, hydrocodone, hydromorphone, buprenorphine, norbuprenorphine, cocaine, benzoylecgonine, cocaethylene, norcocaine, tramadol, fentanyl, amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDEA), nortriptyline, amitriptyline, methadone, diazepam, nordiazepam, oxazepam, alprazolam, chlordiazepoxide, bromazepam, temazepam, lorazepam, flurazepam, nitrazepam, triazolam, tramadol, secobarbital, pentobarbital, butalbital and

phenobarbital to determine any interference. No interference was seen from any of these commonly prescribed drugs.

Ion Suppression / Enhancement. Ion suppression / enhancement was calculated by analyzing 6 neat standard fortifications and 10 extracted blank matrix samples with a post extraction analyte fortification. The post extraction fortification was designed to simulate 100% recovery from the extraction with any interfering matrix. Ion suppression / enhancement was calculated as a percent where the mean area of the post extraction was divided by the mean area of the fortified non-extracted samples, subtracted by 1, then multiplied by 100. This was done at two concentrations, 1 ng/mL and 20 ng/mL designated as low and high, and presented in Table 6.

Analyte	**CBD**	**CBN**	**Δ9-THC**	**Δ8-THC**
Low	-39.41	-27.14	-29.92	-9.63
High	-26.79	-36.13	-40.18	-44.46

Table 6. Ion Suppression / Enhancement calculations drug analytes and corresponding deuterated analogs

Limit of Detection. Limit of detection was determined for each analyte by using the formula 3.3 (y-intercept standard deviation) / (slope standard deviation). This calculation can be used as an estimate and is illustrated in Table 7.

	CBD	**CBN**	**Δ9-THC**	**Δ8-THC**
LOD	0.47	1.09	0.13	0.68

Table 7. Limit of Detection calculations

Dilution Integrity. Samples are routinely screened before confirmation and a general idea of the expected concentration can allow for dilution of the sample prior to extraction. Three dilution factors were analyzed on a 20 ng/mL concentration sample in triplicate over 5 runs. Common dilutions 1:10, 1:5, and 1.2 were used. Mean concentrations and % bias are presented in Table 8.

	CBD	**CBN**	**Δ9-THC**	**Δ8-THC**
(1:10) %Bias	-2.35	-3.16	-2.29	2.86

(1:10) Mean	19.53	19.37	19.54	20.57
(1:5) %Bias	-4.29	-6.59	-6.47	-0.51
(1:5) Mean	19.14	18.68	18.71	19.90
(1:2) %Bias	-4.82	-6.37	-5.56	-1.52
(1:2) Mean	19.04	18.73	18.89	19.70

Table 8. Dilution Integrity % bias and Mean calculations for a 20 ng/mL sample

Conversion of CBD to Δ9-THC and Δ8-THC. Authentic oral fluid samples originally tested for cannabinoids that were suspected of demonstrating conversion of CBD during the initial testing were retested 6 months later using the Cerex Polycrom THC columns and method. Results, compiled in Table 9, showed no conversion of CBD when using the Cerex columns.

Sample ID	**ELISA Screen Results**	**Original Results using Trace-N (ng/mL)**			**Samples extracted using Cerex Polycrom (ng/mL)**		
		CBD	**Δ9-THC**	**Δ8-THC**	**CBD**	**Δ9-THC**	**Δ8-THC**
1	NEG	399	8	2	387	0	0
2	NEG	127	2	1	90	0	0
3	NEG	171	3	0	144	0	0
4	NEG	483	4	1	481	0	0
5	NEG	90	2	4	96	0	0
6	NEG	132	3	2	90	0	0

Table 9. Authentic specimens analyzed 6 months later using Cerex Polycrom THC columns showing no conversion of CBD to Δ9-THC or Δ8-THC.

During the 6-month period, samples were stored in borosilicate glass at refrigerated temperatures. Long-term stability at refrigerated conditions was not assessed for CBD or Δ8-THC in Quantisal collected oral fluid samples. A previous stability study for Δ9-THC reported Quantisal collected oral fluid stable up to 3 months and a loss of 50% after 6 months (11). Based on the potential that Δ9-THC could have degraded over time, expected concentrations for Δ9-THC in the stored samples would still place their concentration within the linear range for testing.

CHAPTER V

CONCLUSION

Section 1:Method Validation Conclusions

Method validation results were considered acceptable by the AAFS Standards Board (ASB) for the following characteristics: bias/precision, calibration, carry over, interference, ion suppression/enhancement, dilution integrity, limit of detection, and limit of quantitation. Improvement for Δ9-THC and Δ8-THC bias and precision could be achieved by better baseline separation. Figure 1 shows they share the same transitions where the peaks overlap and a small portion of the area of integration is shared between them. This can increase the variability of integration.

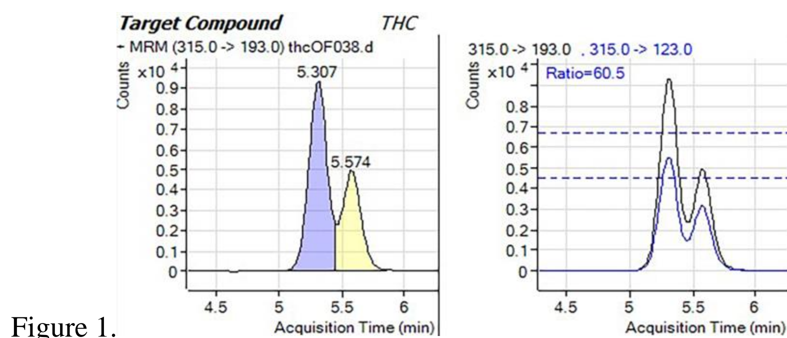

Figure 1.

Conversion of CBD to THC. The purpose of this study was to develop an oral fluid cannabinoid method that would eliminate the conversion of CBD to Δ9-THC and Δ8-THC, and to find the contributing sources causing the conversion. The extraction method using Trace-N columns that was used originally had the conversion rate around 5%. When the pH was kept at 4 and strong

acid washes were removed the rate dropped to 1% on average but was not eliminated. Using the Trace-N method, the time the sample was exposed to acidic conditions was reduced. Initial extracts showed no conversion of CBD; however, during the validation process where larger batches were processed, conversion rates for Δ9-THC and Δ8-THC were around 1.1% and 0.5% respectively. While this was an improvement to previously validated methods, it did not absolutely fix the problem. The Cerex Polycrom THC extraction method did eliminate the conversion of CBD to both Δ9-THC and Δ8-THC. Single analyte CBD controls at 100 ng/mL were monitored and showed no conversion during any of the validation runs. Once validated, the method was used on authentic oral fluid samples, and these also showed no conversion. Figure 2 shows the MRM transitions for sample 4 when processed under the original Trace-N method with CBD conversion and the same sample processed using the Cerex method, with no conversion of CBD present.

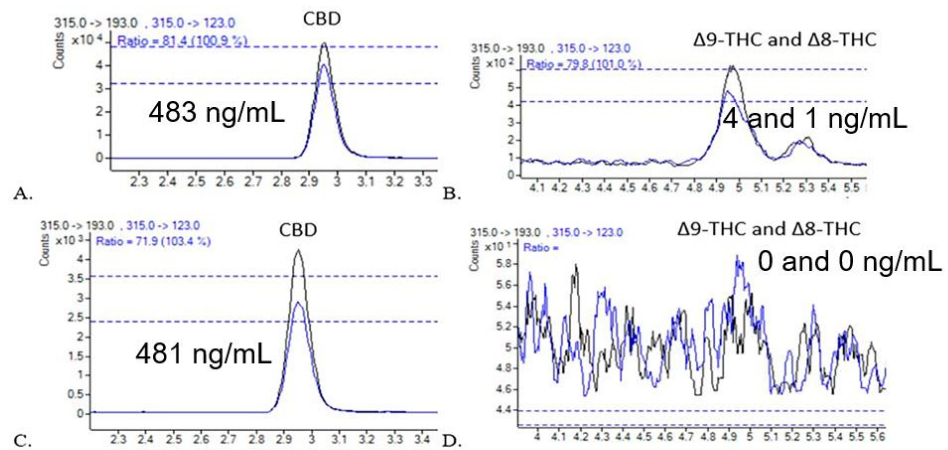

Figure 2.

Oral fluid samples routinely analyzed for cannabinoids showed CBD values between 1 ng/mL – 7028 ng/mL. If a cannabinoid analytical method has a conversion percentage of 1% - 5% then a resulting Δ9-THC concentration could be reported, between 70 ng/mL and 351 ng/mL in a worst-case scenario. Because most labs perform immunoassay based screening prior to confirmation and because CBD's cross reactivity with the screening kit is at 0.04%, equivalent to 10,000

ng/mL, there was a low risk that CBD only samples will be processed (7). An issue may arise where the subject takes both Δ9-THC and CBD. In this case, a high CBD, with a concentration above 5000 ng/mL, may have an additive effect with THC to make a sample appear much higher than the true value. The Cerex Polycrom THC method showed no conversion of CBD to Δ9-THC and Δ8-THC during the validation process, and when tested against authentic samples, making it a viable option when differentiating between THC and CBD concentrations.

Section 2: Limitations of the Method

THC-COOH did not appear to extract at lower concentrations found in oral fluid when using the Cerex method. If the analysis does not require reporting of THC-COOH this could be a viable method for cannabinoid analysis that eliminates CBD conversion. Certified THC-free CBD was used in both dosing prior to sample collection and as a control in method development. Several concentrations for CBD ranging from 1 to 5000 ng/mL were initially tested for conversion. No conversion of CBD to Δ9-THC and Δ8-THC was seen at any level when using the Cerex columns and method. As some sample results were observed that were above 5000 ng/mL, a standalone CBD control near that concentration could be included to ensure no CBD was converted to Δ9-THC and Δ8-THC at very high levels. The concentration of the CBD control should be high enough that a potentially low percentage of conversion would fall in the linear range of the curve. To accurately interpret concentrations found after long-term storage, stability studies would need to be performed on all cannabinoids and potential in vitro conversion during storage would need to be assessed.

The oral fluid cannabinoid method using Cerex Polycrom THC columns validated here eliminates the potential for CBD to convert to Δ9-THC and Δ8-THC *in vitro*. As widespread use and state-wide legalization increase, a clear picture of cannabinoid reporting will become necessary. Avoiding false positive or over/under reporting of Δ9-THC concentrations is critical in a

confirmation method. A mixed analyte, CBD/THC, oral fluid proficiency sample may be a good test for a laboratory's reporting method. By using the method described and validated here, there is less risk the laboratory will see an *in vitro* conversion of CBD to THC. The testing laboratory must put into place safeguards to monitor the potential conversion of CBD, and during method validation they should show that CBD conversion to THC is not taking place due to sample processing and analysis. Further, it is recommended that laboratories use certified THC-free CBD controls in each batch of samples at a high enough concentration to evaluate and monitor potential conversion. . This work was accepted for publication in a peer-reviewed journal (13).

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