ANALYTICAL METHOD FOR THE DETERMINATION OF 15 CANNABINOIDS IN URINE BY UHPLC-MS/MS

INTRODUCTION

Cannabinoid use has increased significantly subsequent to the ratification of the 2018 United States Farm Bill. Cannabinoid products are easily attainable in topical solutions, oral tinctures, edibles, and materials that can be vaporized (vaped) or smoked. A rugged analytical method for the identification and quantitation of cannabinoids is valuable in determining recreational and medical use of these compounds, as well as monitoring potential contaminations in over-the-counter cannabinoid products. As part of a pharmacokinetic study, this analytical method was developed by our laboratory for the determination of 15 different cannabinoids in urine at concentrations from 0.500 to 100 ng/mL

OBJECTIVE

Develop an analytical method for the extraction, detection, and quantitation of (-)- Δ 9-THC, Δ9-Carboxy-THC (Δ9-COOH-THC), 11-Hydroxy-THC (11-OH-THC), Δ9-Tetrahydrocannabivarin (THCV), Δ 9-Carboxy-Tetrahydrocannabivarin (Δ 9-COOH-THCV), (-)- Δ 8-THC, Δ 8-Carboxy-THC (Δ 8-COOH-THC), Cannabidiol (CBD), 7-Hydroxy-Cannabidiol (7-OH-CBD), 7-Carboxy-Cannabidiol (7-COOH-CBD), Cannabidiolic Acid (CBDA), Cannabinol (CBN), Cannabichromene (CBC), Cannabigerol (CBG), and Cannabicyclol (CBL) in urine by LC-MS/MS for a controlled dosing research study.

EXTRACTION METHOD

A 500 μL aliquot of urine specimen and 100 μL of internal standard were combined with 200 μL of Kura BG Turbo β -glucuronidase/0.1M phosphate buffer (pH 6.8) solution in a silanized glass culture tube. Samples were then incubated at 50°C for 15 minutes for hydrolysis. Following this initial hydrolysis step, a secondary hydrolysis was performed with the addition of 100 µL of 5N Potassium Hydroxide to each tube. Samples were vortexed to mix and hydrolyzed at room temperature for 10 minutes, and subsequently 100 μL 5N Formic Acid was added to each tube for neutralization. 1 mL of 0.5% Formic Acid in Acetonitrile was then added to each tube and samples were mixed and poured onto an Agilent Captiva EMR—Lipid 3 mL Cartridge inserted into a silanized glass culture tube. Tubes were centrifuged with cartridges in place to elute the samples. The cartridge was then rinsed with 80:20 Acetonitrile:DI H₂O and again eluted by centrifuging into the same tube. Cartridges were discarded and 1 mL of 0.4M Ammonium Acetate buffer (pH 4.8) and 3 mL of 2:1 Hexanes: Ethyl Acetate were added to the combined eluent. Samples were vortex-mixed for 5 minutes, centrifuged to separate, and placed in a dry ice bath to freeze the aqueous layer. The organic layer was decanted into a silanized glass culture tube and evaporated to dryness under a steady stream of nitrogen at 60°C. For reconstitution, 300 μ L of 0.1% Formic Acid in 50:50 DI H₂O:Methanol was added to each sample and tubes were vortexed for a minimum of 15 seconds.

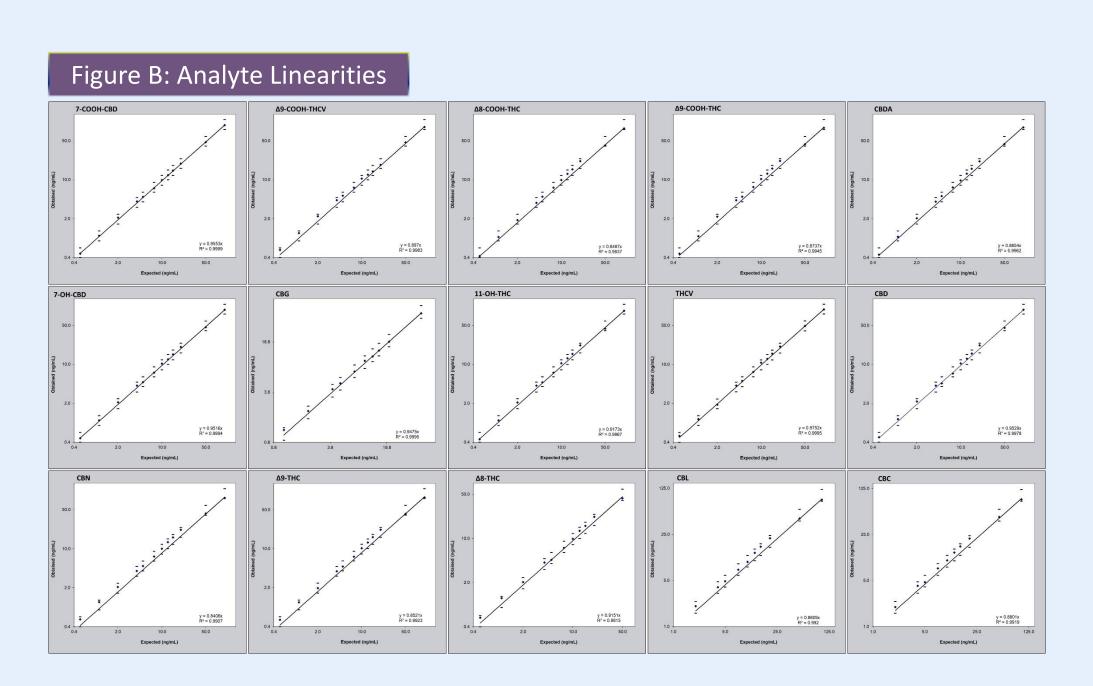
INSTRUMENT PARAMETERS

		LC-40D X3 Pumps	Mass Spectrometer		Sciex API	6500+ Trip	ole Quad		
	SIL-40C X3 Auto Sampler		Ionization	ESI		Positive and Negative			
UHPLC System	Shimadzu Nexera	SCL-40 System Controller	Source Temperature	550°C					
		CTO-40C Column Oven	· · · · · ·	100-sec detection	on window				
		DGU-405 Degassing Unit	Scheduled MRM		Target scan time: 0.100 seconds				
njection Volume	30 μL		Scrieduled Wikivi					Stop: 13.8 mins	
,		008 2.7 2.4		Scheduled Ioni	Scheduled Ionization: Start: 2.8 mins S				
Analytical Column	(Waters Part No. 18600739	90Å, 2.7 μm, 2.1 mm x 150 mm		Positiv	e Ionizati	on			
	•		Analyta	Internal Standard	Trans	itions (±0.3	amu)	Retention Tir	
Guard Column		VanGuard, 90Å, 2.7 μm, 2.1 mm x 5 mm	Analyte	internal Standard	Precursor	Quant	Qual	(± 0.8 minute	
	(Waters Part No. 18600768	35)	11-OH-THC	11-OH-THC-D3	331.2	193.1	201.1	5.83	
Column Temperature	40°C		11-OH-1	THC-D3	334.2	196.1	201.1	5.80	
·	Aqueous:	0.1% Acetic Acid in DI H2O	THCV	CBD-D3	287.3	135.0	123.0	8.53	
Mobile Phase			CBD		318.2	196.2	123.1	8.83	
	Organic:	0.1% Acetic Acid in Acetonitrile	CBD	CBD-D3	315.2	193.2	135.2	8.86	
Flow Rate	0.500 mL/min		CBN	CBN-D3	311.1	241.0	208.0	10.40	
Run Time	15.00 minutes		CBN-		314.1	241.0	208.0	10.37	
	Makila Diana	Condition t	Δ9-THC Δ9-TH	Δ9-THC-D3	315.1 318.1	193.2 196.2	123.0 123.0	11.38 11.34	
100	Mobile Phase	Gradient	Δ8-THC	Δ8-THC-D9	315.1	193.2	135.3	11.65	
			Δ8-TH		324.1	202.2	135.1	11.54	
90 -			CBL	Δ8-THC-D9	315.2	193.0	123.0	12.36	
		· · · · · · · · · · · · · · · · · · ·	CBC	CBC-D3	315.2	193.1	123.1	12.91	
80 -			CBC		318.2	196.1	123.1	12.86	
70				Negati	ve Ionizat	ion			
					Trans	itions (±0.3	amu)	Retention Tir	
60 -			Analyte	Internal Standard	Precursor	<u> </u>	Qual	(± 0.8 minute	
•	0		7-COOH-CBD	7-COOH-CBD-D3	343.2	179.0	231.1	3.51	
50	Time (minutes) Aqueous (9		7-COOH-	CBD-D3	346.2	182.0	234.1	3.49	
40	0.00 53.0 0.50 53.0	47.0 47.0	Δ9-COOH-THCV	7-COOH-CBD-D3	315.2	217.1	163.1	3.78	
40	1.50 52.0	48.0 56.0	Δ8-COOH-THC	Δ8-COOH-THC-D6	343.1	245.1	191.2	5.90	
	4.50 44.0	56.0	Δ8-СООН		349.1	251.1	191.1	5.82	
30	5.50 37.5 9.50 27.5	62.5 72.5	Δ9-COOH-THC	Δ9-COOH-THC-D9	343.1	245.2	191.1	6.13	
			Δ9-СООН	I-THC-D9	352.1	254.1	194.1	6.02	
20 -	12.00 27.5 12.50 15.0	72.5 85.0	CBDA	CBDA-D3	357.2	245.1	227.1	9.02	

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RESULTS / DISCUSSION

Normal human urine fortified with bovine serum albumin was spiked with the 15 cannabinoid analytes at known concentrations and analyzed to establish method linearity and evaluate assay interference and matrix effects. For assay quantitation, a single-point calibrator at 10.0 ng/mL was used. A low control at 4.0 ng/mL (40% of calibrator), two positive controls at 12.5 ng/mL (125% of calibrator), and two negative controls were run with each analytical batch, with one of the negative controls and one of the positive controls injected at the end of the batch to bracket donor samples. In addition to the low and positive controls, every batch included a conversion control and a hydrolysis control. The conversion control was used for monitoring the potential conversion of CBD and its metabolites to $\Delta 9$ -THC and $\Delta 8$ -THC and corresponding metabolites, and contained CBD, 7-OH-CBD, 7-COOH-CBD, and CBDA at 5.0 ng/mL. The hydrolysis control was used to verify that the drug-glucuronide conjugates were sufficiently and consistently hydrolyzing during the extraction process. Because commercially manufactured standards were not available, this control was formulated by pooling specimens that confirmed for the presence of 7-OH-CBD and 7-COOH-CBD by LC-MS/MS; the pooled urine was diluted with certified negative urine to yield CBD-metabolite concentrations within assay linearity, and was then spiked with 11-nor-9-carboxy-Δ9-THC glucuronide to ensure a minimum concentration of 50.0 ng/mL of Δ 9-COOH-THC after hydrolysis.



Linearity was determined and assay limits of detection and quantitation (LOD/LOQ) and upper limit of linearity (ULOL) were established through the analysis of analytespiked samples ranging from 0.500 to 100.0 ng/mL Accuracy and precision were assessed for 5 replicates of each of 12 concentration levels, including 40%, 50%, 100%, 125%, 150%, and 200% of the calibrator. For assay LOD/LOQ, replicates for 7-COOH-CBD, Δ9-COOH-THCV Δ8-COOH-THC, Δ9-COOH-THC, 7-OH-CBD, CBDA, THCV, 11-OH-THC, CBD, CBN, Δ 9-THC, and Δ 8-THC met quantitative acceptability criteria with values within ±20% of target, and met all qualitative acceptance criteria (see Table 2) at the 0.5 ng/mL level. Replicates for CBG met acceptance criteria at 1.0 ng/mL, and CBL and CBC replicates passed at 2.0 ng/mL. At the upper limit of linearity, replicates for all analytes met quantitative and qualitative acceptance criteria at 50.0 ng/mL, while replicates for all analytes with the exception of CBG and $\Delta 8$ -THC passed at 100.0 ng/mL.

Analyte	LOD/LOQ (ng/mL)	Mean at LOD/LOQ	% Mean Accuracy	%CV	ULOL (ng/mL)	Mean at ULOL	% Mean Accuracy	%CV
7-COOH-CBD	0.500	0.470	94.1	3.38	100.0	95.64	95.6	2.29
V9-COOH-THCV	0.500	0.548	109.6	2.15	100.0	88.13	88.1	3.18
Δ8-COOH-THC	0.500	0.426	85.2	4.34	100.0	83.60	83.6	2.44
Δ9-COOH-THC	0.500	0.472	94.4	3.88	100.0	85.75	85.7	3.00
CBDA	0.500	0.452	90.3	4.07	100.0	87.35	87.3	2.90
7-OH-CBD	0.500	0.470	94.0	3.08	100.0	95.14	95.1	3.03
CBG	1.000	1.111	111.1	5.22	50.0	47.167	94.3	2.51
11-OH-THC	0.500	0.453	90.6	4.05	100.0	91.57	91.6	6.56
THCV	0.500	0.523	104.5	9.97	100.0	96.90	96.9	9.56
CBD	0.500	0.490	98.0	4.60	100.0	95.58	95.6	2.50
CBN	0.500	0.542	108.3	1.47	100.0	81.45	81.4	1.27
Δ9-THC	0.500	0.530	105.9	2.28	100.0	83.32	83.3	2.06
Δ8-THC	0.500	0.554	110.8	3.26	50.0	43.014	86.0	2.83
CBL	2.000	2.052	102.6	8.85	100.0	83.87	83.9	3.58
СВС	2.000	1.977	98.9	5.36	100.0	85.09	85.1	3.29

Table 4: Analyte LOD/LOQ and ULOL Accuracy and Precision

Figure C: Representative Chromatogram and Structures of Method Analyte Components in Positive and Negative Ionization Modes **ESI POSITIVE MRM** 11-OH-THC 7-COOH-CBD 7-OH-CBD ESI NEGATIVE MRM Δ9-COOH-THC

Table 2: Quantitative Acceptance Criteria ±2% of expected RRT of the Relative Retention Time (RRT) established by the batch calibrator Asymmetry = <u>B</u> {larger side} A {smaller side} Total IS peak area = nternal Standard (IS) Response ≥10% of calibrator IS peak area Symmetry / Peak Shape asymmetry at 10% of peak height = Time (min) <3.0 for IS and quant peaks Adjacent peaks ≥90% resolved Resolution (≤10% valley/peak height ratio) Ratio of abundance of quantitative Ion Ratios (Qualifiers) to qualifier ion = ±20% of target ratio established by batch calibrator

The potential of sample matrix components to interfere with the analytical method was evaluated by testing ten random negative donor urine samples, extracted unaltered and with cannabinoid analytes spiked at 40% of the calibrator concentration (4.0 ng/mL). Results showed no indication of methodic ion suppression or enhancement, as component recovery was consistent and all spiked samples passed with analyte concentrations within ±20% of target. All samples passed with acceptable chromatography as no qualitative issues were observed, and no interfering peaks were present in the negative samples that could be problematic in quantitation or identification.

Table 3: Evaluation of Matrix Effect Matrix Samples Spiked at 4.0 ng/mL Analytes Std Dev 7-COOH-CBD 4.226 105.61 0.16 9-COOH-THCV Δ9-COOH-THC 113.28 0.24 CBDA 4.533 7-OH-CBD 4.358 108.89 0.22 CBG 4.059 101.43 0.32 11-OH-THC 4.204 105.07 0.29 THCV 4.139 103.44 0.38 CBD 110.11 0.37 4.406 CBN 4.318 107.91 Δ9-THC 4.112 0.28 102.76 Δ8-THC 111.09 0.26 4.445 CBL 4.379 109.44 0.25

Interference was investigated with more than 100 compounds at 5,000 ng/mL each (see Table 5 for complete list). The compounds were spiked in groups into a negative urine sample as well as a urine sample containing the cannabinoid analytes at 40% of calibrator concentration (4.0 ng/mL). Negative samples met acceptance criteria for a negative control, lacking acceptable analyte peak shape and ion ratios and having analyte peak area counts less than 10% of the calibrator. The spiked samples passed all qualitative and quantitative acceptance criteria with analyte values within ±20% of target concentration. Throughout the interference study, no peaks were observed that were greater than the established LOQ for each analyte, which could create possible quantitation or identification issues. No analytical interferences were detected affecting quantitation, chromatography, or indicating ion suppression or enhancement

Table 6: Evaluation of Carryove

Analyte Carryover Check Analyte Peak % of Area Counts Calibrator

Negative after 50 ng/mL 1653.00 0.051				Area Counts	Calibrator Area
Negative after 100 ng/mL 840.00 0.026		7 COOH CBD	Negative after 50 ng/mL	1653.00	0.051
A9-COOH-THCV Negative after 100 ng/mL 2595.00 0.058 Negative after 50 ng/mL 2816.00 0.048 Negative after 100 ng/mL 490.00 0.008 A9-COOH-THC Negative after 50 ng/mL 407.00 0.011 Negative after 100 ng/mL 616.00 0.017 Negative after 50 ng/mL 2564.00 0.017 Negative after 100 ng/mL 5598.00 0.036 Negative after 50 ng/mL 1511.00 0.075 Negative after 100 ng/mL 5057.00 0.562 Negative after 50 ng/mL 5057.00 0.562 Negative after 100 ng/mL 2604.00 0.289 Negative after 50 ng/mL 5335.00 0.187 Negative after 100 ng/mL 9518.00 0.334 Negative after 100 ng/mL 388.00 0.022 CBD Negative after 50 ng/mL 3583.00 0.056 Negative after 50 ng/mL 332.00 0.008 Negative after 50 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101<		/-COOH-CBD	Negative after 100 ng/mL	840.00	0.026
Negative after 100 ng/mL 2595.00 0.058		AO COOH THOV	Negative after 50 ng/mL	1686.00	0.038
A8-COOH-THC Negative after 100 ng/mL 490.00 0.008 A9-COOH-THC Negative after 50 ng/mL 407.00 0.011 Negative after 100 ng/mL 616.00 0.017 Negative after 50 ng/mL 2564.00 0.017 Negative after 100 ng/mL 5598.00 0.036 Negative after 50 ng/mL 5598.00 0.036 Negative after 50 ng/mL 527.00 0.026 Negative after 100 ng/mL 5057.00 0.562 Negative after 50 ng/mL 5057.00 0.289 Negative after 100 ng/mL 9518.00 0.187 Negative after 100 ng/mL 9518.00 0.040 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 808.00 0.022 CBD Negative after 50 ng/mL 3583.00 0.056 Negative after 50 ng/mL 332.00 0.008 Negative after 50 ng/mL 1032.00 0.025 A9-THC Negative after 50 ng/mL 4975.00 0.101 Negative after 50 ng/mL 2363.00 0.04		Д9-СООП-ТПСУ	Negative after 100 ng/mL	2595.00	0.058
Negative after 100 ng/mL 490.00 0.008			Negative after 50 ng/mL	2816.00	0.048
A9-COOH-THC Negative after 100 ng/mL 616.00 0.017 CBDA Negative after 50 ng/mL 2564.00 0.017 Negative after 100 ng/mL 5598.00 0.036 Negative after 50 ng/mL 1511.00 0.075 Negative after 50 ng/mL 527.00 0.026 Negative after 50 ng/mL 5057.00 0.562 Negative after 100 ng/mL 2604.00 0.289 Negative after 50 ng/mL 5335.00 0.187 Negative after 100 ng/mL 9518.00 0.334 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 3583.00 0.056 Negative after 50 ng/mL 332.00 0.034 Negative after 50 ng/mL 4975.00 0.101 Negative after 50 ng/mL 4975.00 0.101 Negative after 50 ng/mL 4975.00 0.048 Negative after 50 ng/mL 4975.00 0.048 Negati		До-СООН-ТПС	Negative after 100 ng/mL	490.00	0.008
Negative after 100 ng/mL			Negative after 50 ng/mL	407.00	0.011
CBDA Negative after 100 ng/mL 5598.00 0.036 7-OH-CBD Negative after 50 ng/mL 1511.00 0.075 Negative after 100 ng/mL 527.00 0.026 Negative after 50 ng/mL 5057.00 0.562 Negative after 100 ng/mL 2604.00 0.289 Negative after 50 ng/mL 5335.00 0.187 Negative after 100 ng/mL 9518.00 0.334 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.0025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038	9	Д3-СООП-ПТС	Negative after 100 ng/mL	616.00	0.017
Negative after 100 ng/mL 5598.00 0.036 Negative after 50 ng/mL 1511.00 0.075 Negative after 50 ng/mL 527.00 0.026 Negative after 50 ng/mL 5057.00 0.562 Negative after 100 ng/mL 2604.00 0.289 Negative after 50 ng/mL 5335.00 0.187 Negative after 100 ng/mL 9518.00 0.334 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 50 ng/mL 332.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 50 ng/mL 1032.00 0.008 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		CRDA	Negative after 50 ng/mL	2564.00	0.017
Negative after 100 ng/mL 527.00 0.026 Negative after 50 ng/mL 5057.00 0.562 Negative after 100 ng/mL 2604.00 0.289 11-OH-THC Negative after 50 ng/mL 5335.00 0.187 Negative after 100 ng/mL 9518.00 0.334 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 50 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 50 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		CDDA	Negative after 100 ng/mL	5598.00	0.036
Negative after 100 ng/mL 527.00 0.026 Negative after 50 ng/mL 5057.00 0.562 Negative after 50 ng/mL 2604.00 0.289 Negative after 100 ng/mL 5335.00 0.187 Negative after 100 ng/mL 9518.00 0.334 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		7-OH-CBD	Negative after 50 ng/mL	1511.00	0.075
CBG Negative after 100 ng/mL 2604.00 0.289 11-OH-THC Negative after 50 ng/mL 5335.00 0.187 THCV Negative after 100 ng/mL 9518.00 0.040 Negative after 50 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 50 ng/mL 4975.00 0.101 Negative after 50 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		7-011-000	Negative after 100 ng/mL	527.00	0.026
Negative after 100 ng/mL 2604.00 0.289		CRG	Negative after 50 ng/mL	5057.00	0.562
THCV Negative after 100 ng/mL 9518.00 0.334 THCV Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 3583.00 0.056 Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		CDG	Negative after 100 ng/mL	2604.00	0.289
Negative after 100 ng/mL 9518.00 0.334 Negative after 50 ng/mL 1462.00 0.040 Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 50 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038 Negative after 50 ng/mL 0.038 Negative af		11-OH-THC	Negative after 50 ng/mL	5335.00	0.187
THCV Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038			Negative after 100 ng/mL	9518.00	0.334
Negative after 100 ng/mL 808.00 0.022 Negative after 50 ng/mL 3583.00 0.056 Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		THCV	Negative after 50 ng/mL	1462.00	0.040
CBD Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		11104	Negative after 100 ng/mL	808.00	0.022
Negative after 100 ng/mL 2173.00 0.034 Negative after 50 ng/mL 332.00 0.008 Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		CBD	Negative after 50 ng/mL	3583.00	0.056
CBN Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		055	Negative after 100 ng/mL	2173.00	0.034
Negative after 100 ng/mL 1032.00 0.025 Negative after 50 ng/mL 4975.00 0.101 Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		CBN	Negative after 50 ng/mL	332.00	0.008
Δ9-THC Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		OBIN	Negative after 100 ng/mL	1032.00	0.025
Negative after 100 ng/mL 2363.00 0.048 Negative after 50 ng/mL 1880.00 0.038		A9-THC	Negative after 50 ng/mL	4975.00	0.101
Δ8-THC		<u> </u>	Negative after 100 ng/mL	2363.00	0.048
		A8-THC	Negative after 50 ng/mL	1880.00	0.038
Negative after 100 ng/mL 1401.00 0.028		<u> </u>	Negative after 100 ng/mL	1401.00	0.028
CBL Negative after 50 ng/mL 1349.00 0.097		CBI	Negative after 50 ng/mL	1349.00	0.097
Negative after 100 ng/mL 1590.00 0.114		GBL	Negative after 100 ng/mL	1590.00	0.114
CBC Negative after 50 ng/mL 293.00 0.014		CBC	Negative after 50 ng/mL	293.00	0.014
Negative after 100 ng/mL 1395.00 0.066		020	Negative after 100 ng/mL	1395.00	0.066

Carryover was tested by injecting negative samples after the 50 and 100 ng/mL ULOL spiked samples. Carryover passed for the negative samples, with no analytes having acceptable peak shape or ion ratios, and analyte peak area counts less than 10% of the calibrator for each compound. The carryover limit was set equal to the ULOL for each analyte.

Table 5: Interfer	ence Compound	s (5,000 ng/mL)
Normeperidine	Pheniramine	Nortripty line
Tramadol	Chlorpheniramine	Norsertraline
ODM-Tramadol	Brompheniramine	Norfluoxetine
Dextromethorphan	Diphenhydramine	Fluoxetine
Pentazocine	Gabapentin	Butalbital
7-Aminonitrazepam	Quetiapine Sulfoxide	Secobarbital
7-Aminoclonazepam	Fentanyl	Phenobarbital
7-Aminoflunitrazepam	Alfentanil	Butabarbital
Hydroxytriazolam	Sufentanil	Amobarbital
Estazolam	Norfentanyl	Pentobarbital
Hydroxyalprazolam	Methadone	Propoxyphene
Nordiazepam	EDDP	Ketamine
Lorazepam	Codeine	Norketamine
2-Hydroxyethylflurazepam	Morphine	Methaqualone
Hydroxymidazolam	Oxycodone	Phenylpropanolamine
Lormetazepam	Oxymorphone	Ephedrine
Oxazepam	Hydrocodone	Pseudoephedrine
Bromazepam	Hydromorphone	Phenylephrine
Temazepam	Norhydrocodone	Phenethylamine
Halazepam	Noroxycodone	Phentermine
Diazepam	6-AM	Acetaminophen
Clonazepam	Dihydrocodeine	Aspirin
Alprazolam	Naltrexone	Ibuprofen
Triazolam	Naloxone	Naproxen
Flurazepam	Nalbuphine	Caffeine
Prazepam	Tapentadol	Hydroxycotinine
Phendimetrazine	Butorphanol	Cotinine
Phenmetrazine	Norbuprenorphine	ETG
Diethylpropion	Buprenorphine	ETS
Ritalinic acid	Cyclobenzaprine	Amphetamine
Meprobamate	Promethazine Sulfoxide	Methamphetamine
Zolpidem	Lamotrigine	MDA
Naltrexol	Aripiprazole	MDMA
Doxylamine	Amitriptyline	MDEA

CONCLUSION

The analytical method reliably identified and quantitated 15 cannabinoids in urine at concentrations from 0.50 to 100 ng/mL, contributing to the scientific knowledge of cannabinoid metabolism and distribution in urine. This method demonstrated selectivity, accuracy, and reproducibility for federally-sponsored research studies.

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DISCLOSURE

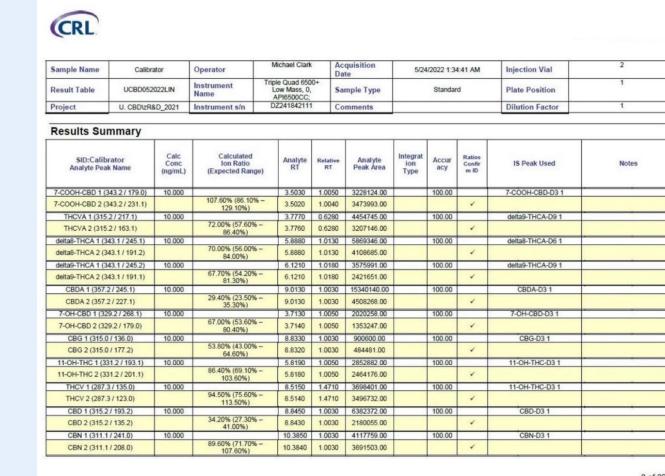


Figure A: Representative CRL MultiQuant Report

2 4 6 Minutes 8 10 12 14

Organic Mobile Phase: 0.1% Acetic Acid in Acetonitrile

SID:Calibrator Analyte Peak Name	Calc Conc (ng/mL)	Calculated Ion Ratio (Expected Range)	Analyte RT	Relati		Analyte eak Area	Integrat ion Type	Accur	Ratios Confir m ID	IS Peak Used	
delta9-THC 1 (315.1 / 193.2)	10.000		11.3640	1.003	30 49	946002.00		100.00		delta9-THC-D3 1	
delta9-THC 2 (315.1 / 123.0)		40.80% (32.70% – 49.00%)	11.3630	1.003	30 20	19630.00			4		
delta8-THC 1 (315.1 / 193.2)	10.000		11.6350	1.009	90 49	941712.00		100.00		delta8-THC-D9 1	
delta8-THC 2 (315.1 / 135.3)		36.90% (29.50% - 44.20%)	11.6350	1.009	90 18	321533.00			1		
CBL 1 (315.2 / 193.0)	10.000		12.3440	1.071	10 13	394248.00		100.00		delta8-THC-D9 1	
CBL 2 (315.2 / 123.0)		116.60% (93.30% - 139.90%)	12.3460	1.071	10 16	25491.00			V		
CBC 1 (315.2 / 193.1)	10.000	130.0070)	12.8880	1.004	10 20	98547.00		100.00		CBC-D3 1	
CBC 2 (315.2 / 123.1)		33.60% (26.90% - 40.30%)	12.8890	1.004	10 7	05428.00			1		
7-COOH-CBD-D3 2 (346.2 / delta9-THCA-D9 1 (352.1 / delta9-THCA-D9 2 (352.1 / delta8-THCA-D6 1 (349.1 /	254.1) 194.1)	107.30% (85.80% 44.50% (35.60%			3.4900 6.0200 6.0200 5.8100	501	18665.0 11547.0 28652.0 73455.0			✓ ✓	
delta8-THCA-D6 2 (349.1/ delta9-THCA-D9 1 (352.1/	The country	48.00% (38.40%	- 57.60%)		5.8100		11547.0			√	
delta9-THCA-D9 2 (352.1/	507 02 v b	44.50% (35.60%	- 53.40%)		6.0200		28652.0			1	
CBDA-D3 1 (360.2 / 248					8.9800	175	25985.0	_			
CBDA-D3 2 (360.2 / 230		30.80% (24.60%	- 36.90%)		8.9800		91130.0			1	
7-OH-CBD-D3 1 (332.2 / 271.1)					3.7000	.7000 2440074					
7-OH-CBD-D3 2 (332.2 / 1	82.0)	65.40% (52.40%	- 78.50%)		3.7000	159	96895.0			V	
CBG-D3 1 (318.0 / 136.	.0)				8.8100	110	0.8880				
CBG-D3 2 (318.0 / 177.	.0)	49.80% (39.80%	- 59.70%)		8.8100	54	7723.0			✓	
11-OH-THC-D3 1 (334.2 / 1	196.1)				5.7900	414	17875.0				
11-OH-THC-D3 2 (334.2 / 2	201.1)	79.10% (63.20%	- 94.90%)		5.7900	327	79096.0			✓	
11-OH-THC-D3 1 (334.2 / 1	196.1)				5.7900	414	17875.0				
11-OH-THC-D3 2 (334.2 / 2	201.1)	79.10% (63.20%	- 94.90%)		5.7900	327	79096.0			V	

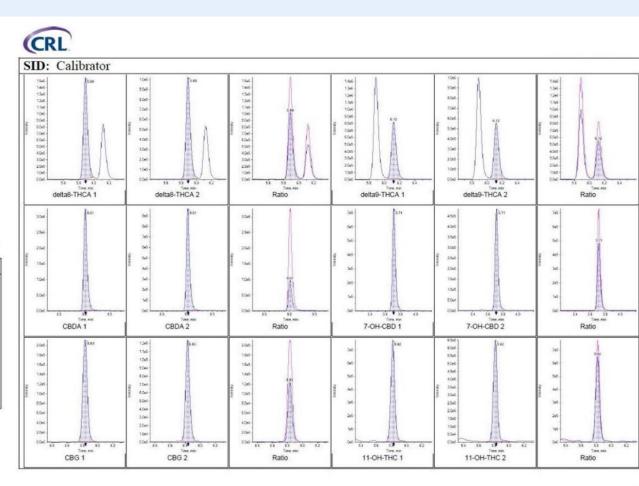
360.2 248.1 230.1 8.98

332.2 271.1 182.0 3.69

318.0 136.0 177.0 8.81

315.0 136.0 177.2

SID:Calibrator Internal Standard Peak Name	Calculated Ion Ratio (Expected Range)	IS RT	IS Peak Area	Integration Type	Ratio Confirms ID	
CBD-D3 1 (318.2 / 196.2)		8.8200	10650583.0			
CBD-D3 2 (318.2 / 135.2)	34.80% (27.80% – 41.70%)	8.8200	3702203.0		✓	
CBN-D3 1 (314.1 / 241.0)		10.3500	5975723.0			
CBN-D3 2 (314.1 / 208.0)	88.50% (70.80% – 106.30%)	10.3500	5291497.0		√	
delta9-THC-D3 1 (318.1 / 196.2)		11.3200	8804138.0			
delta9-THC-D3 2 (318.1 / 123.0)	38.90% (31.10% – 46.70%)	11.3300	3423351.0		√	
delta8-THC-D9 1 (324.1 / 202.2)		11.5300	9219190.0			
delta8-THC-D9 2 (324.1 / 135.1)	38.90% (31.10% - 46.70%)	11.5300	3588132.0		✓	
delta8-THC-D9 1 (324.1 / 202.2)		11.5300	9219190.0			
delta8-THC-D9 2 (324.1 / 135.1)	38.90% (31.10% - 46.70%)	11.5300	3588132.0		V	
CBC-D3 1 (318.2 / 196.1)		12.8300	3279051.0			
CBC-D3 2 (318.2 / 123.1)	5.30% (4.30% - 6.40%)	12.8300	174625.0		✓	
k Review Calibrator						
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	3 300 1 3 4 4 3 3 3 3 3 3 3 3 4 4 4 3 4 3 3 3 4 4 4 3 4 3 4 3 4	156 35 35 35 35 35 35 35 35 35 35 35 35 35	156 3 19 19 156 156 156 156 156 156 156 156 156 156	104 506 806 806 706 606 606 406 106 106 106	156 156 156 156 166 166 166 166 166 166	



SID: Calibrator					
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1040 1040 1040 1040 1040 1040 1040 1040	306 - 306 -	104 106 106 106 106 106 106 106 106	204 254 254 254 254 254 254 254 254 254 25	2560 2560 2560 1560 1560	13-6 10-6 90-6 90-6 90-6 70-6 90-6 40-6 30-6 20-6 10-6

	CRL			CRL
	SID: Calibrator			SID: Calibrator
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112 114 114 114 114 delta8-THC-D9 2	504 456 456 356 356 256 256 166 100 114 Us Us 13 130 132 Time and CBC-D3 1	2000- 2000- 2000- 2000- 2000- 3000- 3000- 3000- 3000- 3000- 300- 2000- 2		,	



No relevant financial or nonfinancial relationships to disclose.