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Quantification of Cannabinoids and their Free and Glucuronide Metabolites in Whole Blood by Disposable Pipette Extraction and Liquid Chromatography Tandem Mass Spectrometry

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Abstract

Identifying recent cannabis intake is confounded by prolonged cannabinoid excretion in chronic frequent cannabis users. We previously observed detection times ≥ 2.1 h for cannabidiol (CBD) and cannabinol (CBN) and THC-glucuronide in whole blood after smoking, suggesting their applicability for identifying recent intake. However, whole blood collection may not occur for up to 4 h during driving under the influence of drugs investigations, making a recent-use marker with a 6–8 h detection window helpful for improving whole blood cannabinoid interpretation. Other minor cannabinoids cannabigerol (CBG), 9-tetrahydrocannabivarin (THCV), and its metabolite 11-nor-9-carboxy-THCV (THCVCOOH) might also be useful. We developed and validated a sensitive and specific liquid chromatography-tandem mass spectrometry method for quantification of THC, its phase I and glucuronide phase II metabolites, and 5 five minor cannabinoids. Cannabinoids were extracted from 200 μ L whole blood via disposable pipette extraction, separated on a C₁₈ column, and detected via electrospray ionization in negative mode with scheduled multiple reaction mass spectrometric monitoring. Linear ranges were 0.5–100 μ g/L for THC and THCCOOH; 0.5–50 μ g/L for 11-OH-THC, CBD, CBN, and THC-glucuronide; 1–50 μ g/L for CBG, THCV, and THCVCOOH; and 5–500 μ g/L for THCCOOH-glucuronide. Inter-day accuracy and precision at low, mid and high quality control (QC) concentrations were 95.1–113% and 2.4–8.5%, respectively (n=25). Extraction recoveries and matrix effects at low and high QC concentrations were 54.0–84.4% and –25.8–30.6%, respectively. By simultaneously monitoring multiple cannabinoids and metabolites, identification of recent cannabis administration or discrimination between licit medicinal and illicit recreational cannabis use can be improved.

Keywords

cannabinoids; whole blood; recent use markers; disposable pipette extraction

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1. Introduction

Cannabis is the most commonly abused drug worldwide [1,2]. Additionally, detection of Δ^9 -tetrahydrocannabinol (THC) in whole blood and/or oral fluid from weekend nighttime drivers increased from 8.6% in 2007 to 12.6% in 2013-2014 [3], furthering public health and safety concerns.

THC and its phase I metabolites 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) are commonly monitored cannabinoids in whole blood by gas chromatography-mass spectrometry (GC-MS) [4-9] or liquid chromatography-tandem MS (LC-MS/MS) [10-17]. However, whole blood THC and THCCOOH can be detected well beyond the window of acute impairment in frequent cannabis smokers [18-20], complicating results interpretation, e.g. identifying recent intake when assessing driving under the influence of drugs (DUID) impairment.

We recently reported that cannabidiol (CBD), cannabinol (CBN) and THC-glucuronide have short detection windows [18,20] and may serve as recent intake markers. However, new cannabinoid plants and plant extracts may have greater CBD concentrations than cannabis included in our previous controlled administration studies, eliminating whole blood CBD as a marker of recent use until pharmacokinetic data are available. Additionally, few analytical methods are available for detection of these analytes in whole blood [12,16].

Cannabigerol (CBG) is a biosynthetic CBD precursor detected in human cannabis users' urine [21]. Δ^9 -Tetrahydrocannabivarin (THCV), a minor cannabis constituent, and 11-nor-9-carboxy-THCV (THCVCOOH) were identified in human urine after cannabis administration [22,23]. The pharmacokinetics of these cannabinoids is poorly characterized, but they may serve as additional markers of recent cannabis intake; to date, there are no methods for their quantification in whole blood.

We developed and validated a LC-MS/MS method for simultaneously quantifying THC, 11-OH-THC, THCCOOH, CBD, CBN, CBG, THCV, THCVCOOH, THC-glucuronide, and THCCOOH-glucuronide in whole blood employing disposable pipette extraction (DPX) tips, which allow for utilization of an automated liquid handler system.

Through simultaneous detection of THC, its phase I and glucuronide phase II metabolites, and 5 minor cannabinoids, identification of recent cannabis administration for DUID investigations, assessing impairment in work or home accidents, and discrimination of licit medicinal from illicit recreational cannabis use can be improved. This method will be employed during our clinical study investigating human performance effects and cannabinoid pharmacokinetics after smoked, vaporized, and oral cannabis administrations to frequent and occasional cannabis smokers; full whole blood pharmacokinetic data will be presented in a future publication.

2. Materials and methods

2.1 Reagents and supplies

THC, 11-OH-THC, THCCOOH, CBD, CBN, THC-d₃, 11-OH-THC-d₃, THCCOOH-d₉, CBD-d₃, CBN-d₃, and THCCOOH-glucuronide-d₃ were purchased from Cerilliant (Round Rock, TX, USA). CBG was from Restek (Bellefonte, PA, USA), THCV was from RTI International (Research Triangle Park, NC, USA), and THCVCOOH and THC-glucuronide were acquired from ElSohly Laboratories (Oxford, MS, USA). Ammonium acetate and acetonitrile (LC-MS grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol and water (LC-MS grade) and formic acid (ACS-grade) were from Fisher Scientific (Fair Lawn, NY, USA). WAX-S tips (1 mL tip containing 20 mg resin and 40 mg salt) were purchased from DPX Labs (Columbia, SC, USA). Chromatography was performed on a Kinetex® C₁₈ column (Phenomenex® Inc., Torrance CA, USA; 2.1 mm × 50 mm, 2.6 µm) combined with a SecurityGuard™ C₁₈ guard column (4 × 2.0 mm).

2.2 Instrumentation

We utilized a Tecan Freedom EVO® 100 liquid handling system (Tecan US Inc., Morrisville, NC, USA), and an HPLC system consisting of a DGU-20A3 degasser, LC-20AD XR pumps, SIL-20AC XR autosampler, and a CTO-20AC column oven (Shimadzu Corp, Columbia, MD, USA) interfaced with a Sciex 5500 QTrap® mass spectrometer with a Turbo V™ ion source (Framingham, MA, USA). Data were acquired and analyzed with Analyst (version 1.5.1) and MultiQuant (version 3.0.1), respectively.

2.3 Calibrators, quality controls, and internal standards

Mixed analyte calibrator solutions were prepared in methanol yielding calibrators at 0.5, 1, 2.5, 5, 10, 25, 50 and 100 µg/L for THC and THCCOOH, at 0.5, 1, 2.5, 5, 10, 25 and 50 µg/L for 11-OH-THC, CBD, CBN, and THC-glucuronide, at 1, 2, 5, 10, 20, 50 and 100 µg/L for CBG, THCV, and THCVCOOH, and at 5, 10, 25, 50, 100, 250 and 500 µg/L for THCCOOH-glucuronide after fortifying 20 µL standard solution in 200 µL whole blood.

Quality control (QC) samples were prepared with reference standards from separate ampules than those used to prepare calibrators. Mixed analyte QC solutions were prepared in methanol and produced QC samples at 1.5, 4.5 and 80 µg/L for THC and THCCOOH, at 1.5, 4.5 and 40 µg/L for 11-OH-THC, CBD, CBN and THC-glucuronide, at 3, 9 and 80 µg/L for CBG, THCV and THCVCOOH, and at 15, 45 and 400 µg/L for THCCOOH-glucuronide when fortifying 20 µL QC solution into 200 µL whole blood.

Mixed internal standard working solution was prepared in methanol containing 50 µg/L THC-d₃, 11-OH-THC-d₃, THCCOOH-d₉, CBD-d₃, and CBN-d₃ and 1000 µg/L for THCCOOH-glucuronide-d₃; fortification volume was 20 µL. There were no commercially available deuterated internal standards for CBG, THCV, THCVCOOH, or THC-glucuronide.

2.4 Disposable pipette extraction

Whole blood specimens (200 µL) were fortified with 20 µL internal standard and proteins precipitated with 500 µL room-temperature acetonitrile. Following thorough vortexing and

centrifugation at 15,000 $\times$ g and 4°C for 5 min, 550 μ L supernatant was transferred to a 2 mL, 96-deep well plate. The plate was transferred to the liquid handling system and 200 μ L 5% aqueous formic acid added to each well, followed by aspiration 4 times through WAX-S tips. These tips contain loosely packed solid-phase sorbent, with which the solution is mixed during sample aspiration. A 1 mL aspiration volume was utilized (an extra 250 μ L air was aspirated to facilitate mixing) at a slow speed. Samples were allowed to mix with the sorbent for 5 s before being dispensed back into the sample tube and aspirated again for a total of 4 aspiration/dispense cycles. Sixty μ L upper, organic layer was transferred to 500 μ L conical glass inserts containing 140 μ L mobile phase A placed in a 96-deep well plate with 1.3 mL round bottom wells, and inserts capped. The plate was vortexed and centrifuged at 700 $\times$ g and 4°C for 5 min before transferring to the autosampler.

2.5 LC-ESI-MS/MS

Chromatographic separation was performed on a Kinetex C₁₈ column via gradient elution with 10mM ammonium acetate in water (A) and 15% methanol in acetonitrile (B). Mobile phase B concentration was initially 30% for 0.5 min, increased to 50% over 0.5 min, to 70.7% over 7.33 min, and to 100% over 0.67 min held for 4.5 min before conditions were returned to 30% B over 0.1 min and held for 2.4 min (total run time 16 min). Flow rate was 0.5 mL/min until 9.00 min, increased to 0.75 mL/min over 0.10 min and held for 4.1 min, and 0.5 mL/min over 0.1 min and held for 2.7 min. Column eluate was diverted to waste for the first 1.2 and final 5 min of analysis. Autosampler and column oven temperatures were set to 4°C and 40°C, respectively.

Data were acquired via negative mode electrospray ionization. The MS was operated in scheduled multiple reaction monitoring (MRM) mode with a 45 s MRM detection window and a 250 ms target scan time, acquiring two MRM transitions for all analytes and internal standards. Optimized MRM settings were determined via 20 μ g/L infusion of each analyte at 10 μ L/min (Table 1).

2.6 Method validation

The method was validated according to the Scientific Working Group for Forensic Toxicology published guidelines [25]. Parameters evaluated include specificity, sensitivity and linearity, accuracy and precision, extraction recovery and matrix effects, carryover, dilution integrity, and stability. Details of these experiments are available in Supplementary Material 1.

2.7 Authentic Specimens

Whole blood specimens were collected from frequent ($\geq 5\times$ /week) and occasional ($\geq 2\times$ /month but $<3\times$ /week) healthy cannabis users who provided written informed consent to participate in a National Institute on Drug Abuse Institutional Review Board-, FDA-, and DEA-approved study. The study was designed, in part, to characterize cannabinoid pharmacokinetics and novel markers of cannabis intake following smoking a single cannabis cigarette containing 6.9% (w/w) THC *ad libitum* within 10 min. Whole blood specimens were collected on admission to the closed clinical unit, within 1 h of dosing (baseline), at the

start of smoking ($t=0.00$ h), every 2 min for the first 12 min after smoking initiation, and up to 72 h after smoking. All blood specimens were stored at -20°C prior to analysis.

3. Results

No endogenous interferences were observed in whole blood from 10 individuals. None of 87 potentially interfering compounds produced low QC concentrations outside of $\pm 20\%$ target or yielded detectable peaks when fortified into negative samples. Only samples fortified with THCV alone and THCCOOH-glucuronide alone produced peaks for other cannabinoids that fulfilled limit of detection (LOD) criteria. In the THCV-only sample (fortified at $100\text{ }\mu\text{g/L}$), 1.7 (1.5% of the fortified concentration) and $1.4\text{ }\mu\text{g/L}$ (1.3% of the fortified concentration) CBD and CBG were observed, respectively. The THCCOOH-glucuronide-only sample fortified at $500\text{ }\mu\text{g/L}$ contained $2.3\text{ }\mu\text{g/L}$ THCCOOH, or 0.5% of the THCCOOH-glucuronide concentration.

Table 3 summarizes linearity results. LODs were $0.25\text{ }\mu\text{g/L}$ for 11-OH-THC, THCCOOH, CBD, CBN, and THC-glucuronide, $0.5\text{ }\mu\text{g/L}$ for THC, CBG, THCV, and THCVCOOH, and $1.25\text{ }\mu\text{g/L}$ for THCCOOH-glucuronide. Linear ranges were $0.5\text{--}100\text{ }\mu\text{g/L}$ for THC and THCCOOH, $0.5\text{--}50\text{ }\mu\text{g/L}$ for 11-OH-THC, CBD, CBN, and THC-glucuronide, $1\text{--}100\text{ }\mu\text{g/L}$ CBG, THCV, and THCVCOOH, and $5\text{--}500\text{ }\mu\text{g/L}$ for THCCOOH-glucuronide; all r^2 were 0.996 employing $1/x^2$ weighting. Scheduled multiple reaction monitoring chromatograms of blank whole blood fortified with analytes at the limit of quantification (LOQ) are presented in Figures 1 and 2.

Intra- and inter-day accuracy ranged from $88.9\text{--}115\%$ and $95.1\text{--}113\%$, respectively and intra- and inter-day precision were $2.4\text{--}6.6\%$ and $2.4\text{--}8.5\%$, respectively (Table 4). One-way ANOVA revealed statistically significant differences in calculated concentrations between batches for several analytes; however, inter-day precisions were 8.5% and considered clinically insignificant.

Recovery and matrix effects for analytes at low and high QC concentrations and internal standards are summarized in Table 5. Recoveries were $54.0\text{--}84.4\%$ and matrix effects were $25.8\text{--}30.6\%$.

No carryover was observed in negative samples following samples fortified at twice each analytes' upper limit of quantification (ULOQ). All analytes quantified within $90.7\text{--}99.7\%$ of target concentrations when diluted 10-fold. After storage at room temperature for 16 h, all analytes quantified within $\pm 20\%$, except for THCVCOOH and THCCOOH-glucuronide. After 72 h at 4°C only THCCOOH-glucuronide failed to quantify within $\pm 20\%$. All analytes quantified within $\pm 20\%$ of target after three freeze-thaw cycles and after 72 h on the 4°C autosampler.

Figures 3 and 4 depict negative and positive authentic samples, respectively, collected following controlled smoked 6.9% THC cannabis.

4. Discussion

We did not observe any interference from potential exogenous compounds fortified at high concentrations (500 µg/L whole blood equivalent). CBD and CBG were detected in a sample fortified with THCV alone at 1.7 and 1.4% of the fortified THCV concentration, respectively. The certificate of analysis for THCV indicates its purity is $94.8 \pm 0.12\%$, indicating that CBD and CBG may be present within the standard. These data agree with results obtained during method development conducted by our laboratory for cannabinoids quantification in oral fluid, in which the same THCV standard was utilized; CBD and CBG were detected in samples fortified with THCV alone at 1.5 and 1.3% of THCV concentration, respectively [26]. Additionally, THCCOOH was detected in a sample fortified with THCCOOH-glucuronide at 0.5% of fortified THCCOOH-glucuronide concentration. This agrees with findings from development of our previous method quantifying cannabinoids in whole blood [12], in which it was determined the THCCOOH-glucuronide standard contained $0.5 \pm 0.1\%$ THCCOOH; the same THCCOOH-glucuronide standard was utilized in the present method. Overall, the method demonstrated good selectivity for all analytes (Figures 1-4).

During method development, we attempted to include Δ^9 -tetrahydrocannabinolic acid A (THCAA), THC's biosynthetic precursor because it was previously detected in serum [27,28], plasma and whole blood [29] from cannabis users, and may serve as a marker of illicit or recent cannabis administration. During validation, however, intra- and inter-day precision were 13.6-28.6%, intra-day accuracies ranged from 76.1-138%, and large ion suppression (56.2-63.2%) was observed. Deuterated THCAA is not commercially available; previous methods quantified THCAA utilizing mismatched deuterated internal standards [27,30], or a custom-synthesized THCAA- d_3 internal standard [28,29]. We attempted to utilize THCCOOH- d_9 as an internal standard because it eluted closest to THCAA in the chromatographic gradient. The ion suppression observed for THCCOOH- d_9 (18.4-18.6%) was not comparable to that of THCAA (56.2-63.2), and the suppression observed for THCAA was not reproducible between 10 different whole blood lots (%RSD = 30%), yielding unacceptable accuracy and precision; preventing its inclusion in our method. THCAA may serve as a useful marker of illicit or recent cannabis use, but monitoring this analyte will not be feasible for most forensic laboratories until a matched, deuterated internal standard becomes commercially available.

Utilization of DPX tips permitted simultaneous extraction and quantification of five parent cannabinoids and five metabolites, including glucuronides, via LC-MS/MS. Extraction efficiency via DPX differs from solid phase extraction (SPE) methods because it is based on the equilibration time after mixing of the sample with the sorbent – which maximizes interactions with the sorbent – rather than flow rate. Additionally, extraction with DPX WAX-S tips requires no sorbent conditioning or washing with analytes extracted into the organic phase remaining in the sample tube, a process that is easily automated. Overall, sensitive LOQs were achieved and matrix effects were minimized after implementing DPX WAX-S tips, which are particularly important considerations when extracting cannabinoids from whole blood.

Previous methods quantifying cannabinoids in whole blood with LC-MS/MS monitored THC, 11-OH-THC, and THCCOOH [10,12,14,15,17]. In comparison, we had comparable or better recoveries and matrix effects for at least one of these analytes. Our laboratory's previous whole blood cannabinoid method [12] is the only published method with quantitative data for the recovery and matrix effects of CBN, CBD, THC-glucuronide, and THCCOOH-glucuronide after solid-phase extraction. By implementing DPX tips for extraction, we achieved similar recoveries but improved matrix effects for these four analytes. As a result, all analytes could be quantified accurately and reproducibly with high sensitivity (LOQs 0.5-5 µg/L) and linear ranges that are clinically relevant, minimizing repeat analyses to dilute samples into the linear range. This method is applicable for a variety of testing environments and improves overall analysis productivity through decreased sample preparation time.

DPX tips were previously utilized to quantify THC in whole blood [8] and THCCOOH in urine [8,24]. The tips implemented required sample mixing with the sorbent, followed by washing and elution steps, similar to solid-phase extraction performed with sorbent packed into a cartridge. Extraction with the WAX-S tips implemented in this method did not require separate wash and elution steps, as matrix interferences, rather than analytes, interact with and are retained on the sorbent or are retained in the aqueous phase while analytes are extracted into the organic phase. After sample aspiration, a phase separation occurs in which analytes are extracted into the upper organic layer, a portion of which is removed for analysis. Therefore, utilization of the WAX-S tips compared to those with ion-exchange mechanisms decreased sample analysis time and reduced solvent use, while still achieving low LOQs with small matrix effects.

This is the first method to our knowledge that quantifies THCV, THCVCOOH, and CBG in whole blood. THCV was previously quantified in rat and mouse plasma following intraperitoneal and oral administrations [31]. To date, the only available data regarding the detection of these analytes in humans after cannabis use are in urine. It was previously demonstrated that THCV was not present in synthetic dronabinol preparations [32]. Additionally, THCVCOOH was present in human urine only after participants smoked cannabis cigarettes containing THCV but not after oral dronabinol [22]. More recently, the prevalence of THCVCOOH in human urine was determined as part of a study investigating the efficacy of dronabinol for the treatment of cannabis dependence and was detected in 50% of admission urine specimens [23]. CBG also was detected in human urine after cannabis use [21]. Inclusion of these analytes in whole blood analyses can aid in cannabinoid results interpretation because they can help rule out medical dronabinol use and may be markers of recent cannabis use.

5. Conclusion

We present a novel LC-MS/MS method for the quantification of 10 cannabinoids and free and glucuronide metabolites in whole blood, utilizing DPX tips for simultaneous extraction of all analytes with high sensitivity (LOQs 0.5-5 µg/L). This method was validated with clinically relevant linear ranges limiting repeat analyses for concentrated samples, making it applicable for forensic and clinical testing. By implementing DPX tips for extraction,

analysis time was decreased and sample throughput increased. This method can aid whole blood cannabinoid results interpretation by monitoring the most comprehensive panel of major and minor cannabinoids and metabolites to date that may improve identification of recent cannabis intake or distinguish licit medicinal and illicit cannabis administration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Quantification of multiple blood cannabinoids improves results interpretation
- Disposable pipette extraction allows for sensitive cannabinoid quantification
- Five parent cannabinoids and 5 metabolites were simultaneously extracted
- Cannabinoids were quantified via LC-MS/MS with clinically-relevant linear ranges
- This method is applicable for forensic and clinical drug testing programs

Figure 1.

Scheduled multiple reaction monitoring ion chromatograms for quantifier transitions illustrate analyte peaks distinguishable from noise at the limits of quantification. *THC* ⁹-tetrahydrocannabinol, *11-OH-THC* 11-hydroxy-THC, *THCCOOH* 11-nor-9-carboxy-THC, *CBD* cannabidiol, *CBN* cannabinol, *CBG* cannabigerol, *THCV* ⁹-tetrahydrocannabivarin, *THCVCOOH* 11-nor-9-carboxy-THCV.

Figure 2.

Scheduled multiple reaction monitoring ion chromatograms for quantifier transitions in blank whole blood at limits of quantification demonstrate chromatographic resolution between target analytes. *THC* ⁹-tetrahydrocannabinol, *11-OH-THC* 11-hydroxy-THC, *THCCOOH* 11-nor-9-carboxy-THC, *CBD* cannabidiol, *CBN* cannabinol, *CBG* cannabigerol, *THCV* ⁹- tetrahydrocannabivarin, *THCVCOOH* 11-nor-9-carboxy-THCV.

Figure 3.

Scheduled multiple reaction monitoring ion chromatograms for quantifier transitions from an authentic negative specimen collected from an occasional cannabis smoker 54 h after smoking a 6.9% THC cigarette demonstrates chromatographic selectivity. *THC*⁹-tetrahydrocannabinol, *11-OH-THC* 11-hydroxy-THC, *THCCOOH* 11-nor-9-carboxy-THC, *CBD* cannabidiol, *CBN* cannabinol, *CBG* cannabigerol, *THCV*⁹-tetrahydrocannabivarin, *THCVCOOH* 11-nor-9-carboxy-THCV.

Figure 4.

Scheduled multiple reaction monitoring ion chromatograms for quantifier transitions from authentic positive specimens collected from a frequent cannabis smoker 0.13 h (0.5 h for THC-glucuronide) after smoking a 6.9% THC cigarette demonstrate chromatographic selectivity. *THC* ⁹-tetrahydrocannabinol, *11-OH-THC* 11-hydroxy-THC, *THCCOOH* 11-nor-9-carboxy-THC, *CBD* cannabidiol, *CBN* cannabinol, *CBG* cannabigerol, *THCV* ⁹-tetrahydrocannabivarin, *THCVCOOH* 11-nor-9-carboxy-THCV.

Table 1

Unique tandem mass spectrometry parameters for 10 cannabinoids in whole blood

Analyte	Q1 mass (<i>m/z</i>)	Q3 mass (<i>m/z</i>) ^a	DP (V)	CE (V)	CXP (V)	RT (min)
THC	313.1	245.1	−170	−36	−21	7.83
		191.1		−36	−15	
11-OH-THC	329.0	268.1	−150	−34	−23	4.08
		173.0		−40	−19	
THCCOOH	343.0	245.0	−110	−38	−23	2.48
		191.0		−44	−17	
CBD	313.0	179.1	−135	−24	−9	5.92
		245.0		−30	−23	
CBN	309.0	279.0	−135	−42	−23	7.14
		221.9		−64	−19	
CBG	315.2	136.0	−50	−34	−17	6.07
		190.9		−32	−17	
THCV	285.0	216.9	−155	−40	−23	5.55
		162.8		−30	−21	
THCVCOOH	315.0	271.1	−130	−28	−21	1.85
		217.0		−36	−19	
THC-glucuronide	489.1	313.2	−20	−42	−31	2.00
		175.0		−26	−19	
THCCOOH-glucuronide	519.2	343.0	−15	−32	−31	1.94
		299.1		−46	−27	
THC-d3	316.1	248.1	−170	−38	−27	7.82
		194.0		−42	−19	
11-OH-THC-d3	332.0	271.1	−125	−38	−23	4.07
		173.0		−46	−11	
THCCOOH-d9	352.2	254.1	−150	−38	−23	2.47
		195.0		−40	−17	
CBD-d3	316.0	182.1	−120	−26	−5	5.91
		248.1		−32	−19	
CBN-d3	312.0	282.1	−165	−42	−27	7.13
		222.0		−56	−19	
THCCOOH-glucuronide-d3	522.0	346.2	−135	−30	−27	1.93
		302.2		−50	−33	
Optimized Source Settings						
Spray Voltage			−4.5 kV			
Gas-1			345 kPa			
Gas-2			379 kPa			
Curtain Gas			310 kPa			
Source Temperature			550 °C			
Mass Resolution			Unit (Q1 & Q3)			

Analyte	Q1 mass (<i>m/z</i>)	Q3 mass (<i>m/z</i>) ^a	DP (V)	CE (V)	CXP (V)	RT (min)
Nitrogen Collision Gas			Medium			
Entrance Potential			−10 V			

Q1 quadrupole 1, *Q3* quadrupole 3, *DP* declustering potential; *CE* collision energy, *CXP* collision cell exit potential, *RT* retention time, *THC* ⁹-tetrahydrocannabinol, *11-OH-THC* 11-hydroxy-THC, *THCCOOH* 11-nor-9-carboxy-THC, *CBD* cannabidiol, *CBN* cannabinol, *CBG* cannabigerol, *THCV* ⁹-tetrahydrocannabivarin, *THCVCOOH* 11-nor-9-carboxy-THCV

^aBolded ions utilized for quantification

Table 2

Eighty-seven exogenous interferences were evaluated at 500 µg/L whole blood equivalent concentrations and did not produce cannabinoid concentrations outside $\pm 20\%$ target when fortified into low QC samples or yield detectable peaks when fortified into negative (internal standard only) samples.

Cocaine	Opioids	Benzodiazepines	Amphetamines and other amines	Antidepressants	Others
cocaine	morphine	diazepam	<i>p</i> -methoxyamphetamine	imipramine	clonidine
benzoylcegonine	normorphine	lorazepam	<i>p</i> -methoxymethylamphetamine	clomipramine	ibuprofen
norcocaine	morphine-3- β -D-glucuronide	oxazepam	methamphetamine	fluoxetine	caffeine
norbenzoylcegonine	morphine-6- β -D-glucuronide	alprazolam	amphetamine	norfluoxetine	diphenhydramine
ecgonine ethyl ester	codeine	7-aminoclonazepam	hydroxylamphetamine	paroxetine	chlorpheniramine
ecgonine methyl ester	norcodeine	7-aminoflunitrazepam	hydroxylmethamphetamine		brompheniramine
anhydroecgonine methyl ester	6-acetylmorphine	7-aminonitrazepam	4-hydroxy-3-methoxymethamphetamine		Aspirin
ecgonine	6-acetylcodeine	nitrazepam	4-hydroxy-3-methoxyamphetamine		Tylenol
cocaehtylene	buprenorphine	flunitrazepam	3,4-methylenedioxymphetamine		ketamine
norocaethylene	norbuprenorphine	temazepam	3,4-methylenedioxymethamphetamine		dextromethorphan
<i>m</i> -hydroxycocaine	hydrocodone	nordiazepam	3,4-methylenedioxyethylamphetamine		nicotine
<i>p</i> -hydroxycocaine	hydromorphone	bromazepam	3,4-methylenedioxyphenyl-2-butanamine		cotinine
<i>m</i> -hydroxybenzoylcegonine	oxycodone	clonazepam	methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine		norcotinine
<i>p</i> -hydroxybenzoylcegonine	noroxycodone	flurazepam	R-cathinone		hydroxycotinine
	oxymorphone		ethylamphetamine		
	noroxymorphone		4-bromo-2,5-dimethoxyphenethylamine		
	methadone		ephedrine		
2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine			pseudoephedrine		
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine			phentermine		
propoxyphene					
pentazocine					

Table 3

Sensitive limits of detection (LOD) and quantification (LOQ) and clinically-relevant linear calibration ranges were achieved for cannabinoids in whole blood (n=5)

Analyte	Internal Standard	LOD (µg/L)	LOQ (µg/L)	Linear Range (µg/L)	Mean y-intercept ± SD	Mean slope ± SD	r ² (range)
THC	THC-d ₃	0.5	0.5	0.5-100	0.002 ± 0.014	0.226 ± 0.005	0.996-1.000
11-OH-THC	11-OH-THC-d ₃	0.25	0.5	0.5-50	0.001 ± 0.007	0.191 ± 0.004	0.999-0.999
THCCOOH	THCCOOH-d ₉	0.25	0.5	0.5-100	0.004 ± 0.009	0.167 ± 0.064	0.998-0.999
CBD	CBD-d ₃	0.25	0.5	0.5-50	0.007 ± 0.006	0.243 ± 0.005	0.997-1.000
CBN	CBN-d ₃	0.25	0.5	0.5-50	-1.02 ± 2.30	0.191 ± 0.003	0.998-1.000
CBG	CBD-d ₃	0.5	1.0	1-50	0.006 ± 0.008	0.090 ± 0.005	0.997-0.999
THCV	CBD-d ₃	0.5	1.0	1-50	-0.001 ± 0.004	0.057 ± 0.002	0.999-1.000
THCVCOOH	THCCOOH-d ₉	0.5	1.0	1-50	-0.050 ± 0.027	0.774 ± 0.294	0.997-0.999
THC-glucuronide	THCCOOH-glucuronide-d ₃	0.25	0.5	0.5-50	0.003 ± 0.002	0.055 ± 0.012	0.997-0.999
THCCOOH-glucuronide	THCCOOH-glucuronide-d ₃	1.25	5.0	5-500	0.016 ± 0.008	0.022 ± 0.001	0.998-0.999

THC ⁹-tetrahydrocannabinol, 11-OH-THC 11-hydroxy-THC, THCCOOH 11-nor-9-carboxy-THC, CBD cannabidiol, CBN cannabinol, CBG cannabigerol, THCV ⁹-tetrahydrocannabivarin, THCVCOOH 11-nor-9-carboxy-THCV

Table 4

Extraction via disposable pipettes with liquid chromatography tandem mass spectrometry detection yielded reproducible and accurate cannabinoid quantification in whole blood.

Analyte	Intra-day precision (%RSD, n=5/day)			Inter-day precision (%RSD, n=25)			Accuracy (% of target, n=25)		
	Low	Mid	High	Low	Mid	High	Low	Mid	High
THC ^a	5.9	4.2	4.3	6.8	5.0	5.2	108	107	105
11-OH-THC ^a	5.8	3.4	3.0	5.8	6.5	3.9	104	103	104
THCCOOH ^a	6.3	3.6	3.6	6.5	6.2	3.5	105	105	104
CBD ^a	3.9	3.7	3.3	4.0	5.5	3.6	109	108	111
CBN ^a	4.7	2.9	2.4	4.7	3.9	2.4	111	109	113
CBG ^b	4.2	5.4	4.1	5.5	6.9	5.0	102	105	108
THCV ^b	4.1	3.4	3.5	5.7	6.7	4.9	106	106	108
THCVCOOH ^b	4.8	5.4	3.1	7.4	8.5	4.3	96.0	95.1	106
THC-glucuronide ^a	6.6	5.9	3.9	8.3	6.1	4.0	101	105	96.7
THCCOOH-glucuronide ^c	3.9	4.2	2.5	4.1	4.7	3.1	103	104	96.9

THC^a 9-tetrahydrocannabinol, 11-OH-THC 11-hydroxy-THC, THCCOOH 11-nor-9-carboxy-THC, CBD cannabidiol, CBN cannabinol, CBG cannabigerol, THCV^b 9-tetrahydrocannabivarin, THCVCOOH 11-nor-9-carboxy-THCV

^aLow-, and mid-quality control concentrations for THC, 11-OH-THC, THCCOOH, CBD, CBN, and THC-glucuronide were 1.5 and 4.5µg/L, respectively. High-quality control concentration for THC and THCCOOH was 80µg/L and for 11-OH-THC, CBD, CBN, and THC-glucuronide it was 40µg/L

^bLow-, mid-, and high-quality control concentrations for CBG, THCV, and THCVCOOH were 3, 9, and 80µg/L, respectively

^cLow-, mid-, and high-quality control concentrations for THCCOOH-glucuronide were 15, 45, and 400µg/L, respectively

Table 5

Extraction via disposable pipettes yielded efficient recoveries with minimal matrix effects enabling sensitive limits of quantification (0.5-5.0 µg/L).

Analyte	Recovery (%; n=10)		Matrix Effect (%; n=10)	
	Low	High	Low	High
THC ^a	59.9	58.7	-23.0	-21.5
11-OH-THC ^a	78.6	79.5	-6.1	-4.8
THCCOOH ^a	71.3	72.1	-17.5	-20.6
CBD ^a	73.0	73.6	-6.5	-6.1
CBN ^a	54.0	55.4	-11.0	-14.1
CBG ^b	63.5	65.5	-10.8	-13.0
THCV ^b	66.4	69.0	30.6	23.9
THCVCOOH ^b	70.1	75.3	7.7	3.5
THC-glucuronide ^a	71.3	72.2	4.5	1.1
THCCOOH-glucuronide ^c	55.4	56.4	2.2	-1.4
THC-d ₃	65.2	64.9	-25.8	-25.7
11-OH-THC-d ₃	84.4	83.2	-14.3	-10.4
THCCOOH-d ₉	75.2	75.5	-18.4	-18.6
CBD-d ₃	81.4	80.6	-13.7	-8.4
CBN-d ₃	59.2	59.7	-16.2	-15.9
THCCOOH-glucuronide-d ₃	58.8	59.3	-3.3	-5.5

THC⁹-tetrahydrocannabinol, 11-OH-THC 11-hydroxy-THC, THCCOOH 11-nor-9-carboxy-THC, CBD cannabidiol, CBN cannabinol, CBG cannabigerol, THCV⁹-tetrahydrocannabivarin, THCVCOOH 11-nor-9-carboxy-THCV

^aLow-quality control concentration for THC, 11-OH-THC, THCCOOH, CBD, CBN, and THC-glucuronide was 1.5µg/L. High-quality control concentration for THC and THCCOOH was 80µg/L and for 11-OH-THC, CBD, CBN, and THC-glucuronide it was 40µg/L

^bLow- and high-quality control concentrations for CBG, THCV, and THCVCOOH were 3 and 80µg/L, respectively

^cLow- and high-quality control concentrations for THCCOOH-glucuronide were 15 and 400µg/L, respectively