Whole Blood Analysis of Δ9 Tetrahydrocannabinol and its Two Main Metabolites Using Automated Dispersive Pipette XTRaction and LC-MS/MS

HIGHLIGHTS: THC Extraction in < 2 minutes

WAX-S - XTR

INTRODUCTION

Cannabis is the most widely abused illicit drug in the US. Results from the 2013 National Survey on Drug Use and Health show that between 2007 and 2012 the rate of marijuana use rose from 5.8% to 7.3%. As use of marijuana increases, it becomes more prevalent in clinical and forensic case work, particularly in impaired driving cases. In 2007 the National Highway Traffic Safety Administration (NHTSA) reported that 8.6% of nighttime drivers tested positive for cannabinoids—a rate almost four times higher than those with blood alcohol levels equal to or above 0.8 g/L. Recent marijuana use is associated with a 2–6 fold increased crash risk, depending on dose, than when unimpaired.

Δ9-Tetrahydrocannabinol (THC) is the main psychoactive ingredient in cannabis (marijuana). After smoking, THC is rapidly absorbed into the blood stream. THC is metabolized into two main metabolites, the active metabolite, 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC), and the inactive metabolite, 11-nor-9-carboxy-Δ9 -tetrahydrocannabinol (THC-COOH). THC affects mental processes, ranging from altered perception of time and distance to hallucinations, owing to the risk of use while driving. Peak THC effects are 20–30 minutes after use, when blood levels are highest, concentrations continue to fall becoming baseline after 4 hours.

Blood is routinely the matrix of choice when determining drug or alcohol impairment. Colorado and Washington have legalized recreational marijuana use and adopted a driving under the influence of drugs (DUID) per se blood threshold of 5 ng/mL THC. Unfortunately, blood draws are not done at the scene and can take up to hours after the time of the incident. Detection and quantification of THC and its metabolites in blood at low levels (< 5 ng/mL) are imperative in determining time of THC use and potential impairment.

Sample preparation was performed on a Hamilton Microlab Nimbus96

METHOD

Sample aliquots of 100 µL were transferred to a 2 mL micro centrifuge tube. Internal standard in methanol was added (10 µL) and the tubes were vortex mixed. Acetonitrile (300 µL) was added and the tubes were vortexed and centrifuged for 10 min at 13,300 rpm. The supernatant (350 µL) was transferred to a 2.2 mL well plate, which was then placed on a Hamilton NIMBUS96® system for the automated solid-phase extraction procedure (for increased efficiency, see the DPX Tip-on-Tip Technical Note). The NIMBUS® was loaded with XTR tips, 300 µL CO-RE tips, a reservoir of 0.1M formic acid, and an additional empty well plate. The NIMBUS uses the CO-RE tips to add 50 µL of 0.1M formic acid to the well plate containing the sample supernatant. XTR tips containing 10 mg WAX + 40 mg Salt (WAX-S) are then used to aspirate and dispense the sample solution four times. This step allows for extraction of matrix and subsequent partitioning of the acetonitrile and aqueous phases. The CO-RE tips are used to transfer 100 µL of the acetonitrile layer to a clean well plate, which is then transferred to the LC-MS/MS for injection.

1	Blood + I.S. + ACN Vortex & Centrifuge (for increased efficiency see ToT Protein Precipitation Tech Note.)	
2	Transfer Supernatant to well plate	
3	Load Nimbus96 Deck	
4	Click Play	
	A	Add 0.1 M FA to Sample Solution
	B	Aspirate/Dispense 4X with WAX-S - XTR tips (See Schematic)
	C	Transfer Supernatant
5	Inject onto LC-MS/MS	

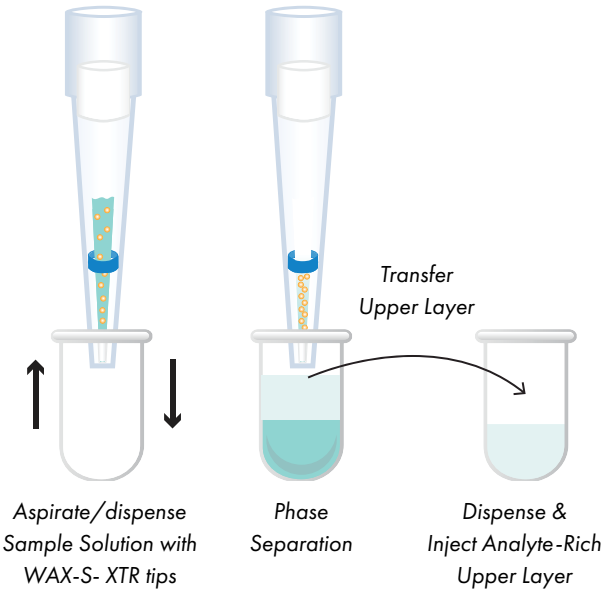

Figure 1. Visual representation of Dispersive Pipette XTRaction + SALLE (salt-assisted liquid-liquid extraction). The salt dissolves in solution during aspirate/dispense steps and assists in phase separation. The analyte-rich, acetonitrile upper layer in the final solution, now ready for analysis.

RESULTS AND DISCUSSION

Linear regression analysis resulted in average coefficient of determination (R^2) values of 0.9984 for THC, 0.9980 for 11-OH-THC, and 0.9966 for THC-COOH. The average slope (Avg_m) and the standard deviation of the y-intercept (S_y) values were used to determine the limits of detection (LODs) and limits of quantitation (LOQs) for each compound (0.66 ng/mL for THC, 0.75 ng/mL for 11-OH-THC, and 1.8 ng/mL for THC-COOH) as shown in Table 1. The LOQ for each compound was well below the recommended DUID confirmation cut-off concentration in blood of 1, 5, and 1 ng/mL for THC, THC-COOH, and 11-OH-THC, respectively.

Table 1. Standard deviation of the y-intercept (Sy), average slope (Avgm , N=5), limit of detection (LOD, ng/mL), limit of quantitation (LOQ, ng/mL), and average coefficient of determination (R2, N=5)

Drug	S_y	Avg_m	LOD	LOQ	Avg R^2
THC	0.001	0.014	0.22	0.67	0.9984
11-OH-THC	0.006	0.074	0.25	0.75	0.9980
THC-COOH	0.003	0.016	0.62	1.8	0.9966

Table 2 illustrates accuracy, within-run precision and between-run precision data. The method was very accurate with a minimum bias of 0.2% at 25 ng/mL 11-OH-THC and a maximum of 4.7% at 2 ng/mL THC. Within-run and between-run precision were determined from the same replicate analyses. Average maximum within-run precision was 6.8% at 2 ng/mL THC with maximum between-run precision of 7.5% at 2 ng/mL THC.

Low analyte concentrations are most susceptible to large matrix effects. Ion suppression for THC was 31% at 5 ng/mL, but only 12% at 20 ng/mL. 11-OH-THC elicited ion suppression at 1 ng/mL of 6% and 2% at 2 ng/mL. THC-COOH had negligible matrix effects, showing ion suppression at 10 ng/mL of 3% but ion enhancement at 40 ng/mL of 3%. Dispersive pipette extraction was very efficient at >93% at both concentrations for all compounds. Analyte loss during the protein precipitation step also varied with concentration. There was approximately 30% loss of all compounds at 25 ng/mL, but 15%, 19%, and 8% of THC, 11-OH-THC and THC-COOH, respectively, were lost at 2 ng/mL.

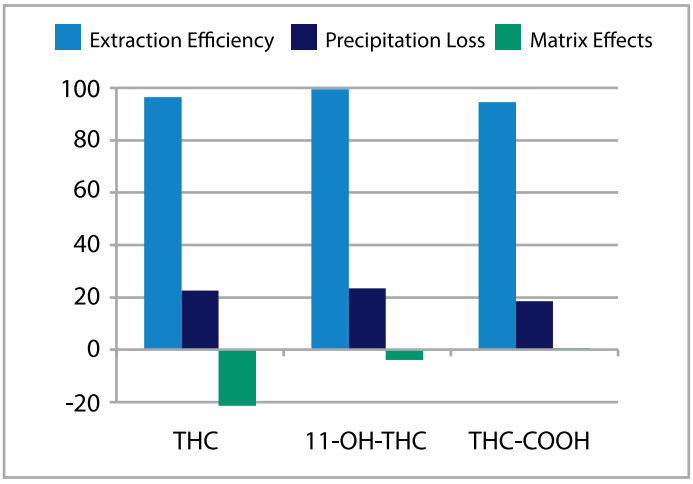

Figure 3. Average extraction efficiency, precipitation loss, and matrix effects (%) for THC, 11-OH-THC, and THC-COOH.

Table 2. Accuracy, within-run precision, and between-run precision calculated according to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

Accuracy (%)			
	2 ng/mL	10 ng/mL	25 ng/mL
THC	104.7	99.3	99.8
11-OH-THC	98.7	97.2	100.2
THC-COOH	96.4	99.3	99.6
Within-Run precision (%)			
	2 ng/mL	10 ng/mL	25 ng/mL
THC	6.8	4.6	2.8
11-OH-THC	5	3.5	1.8
THC-COOH	6.4	4.5	3
Between-Run Precision (%)			
	2 ng/mL	10 ng/mL	25 ng/mL
THC	7.5	5.6	2.9
11-OH-THC	6.6	6	3.5
THC-COOH	7	4.4	3.1

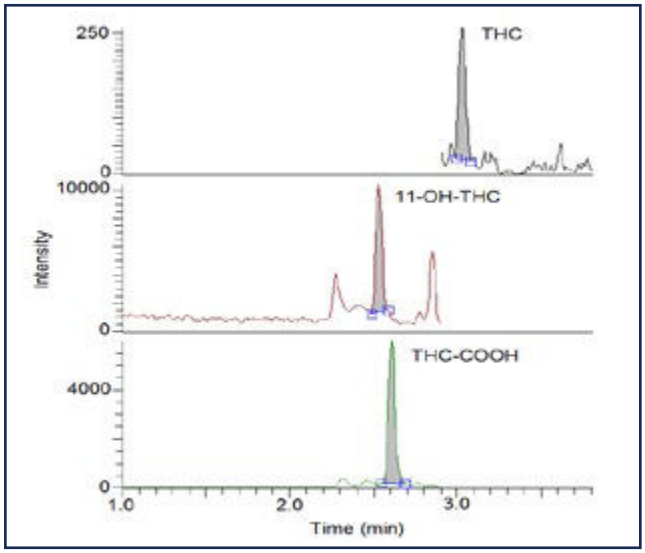

Figure 2. Patient Sample 10: positive for 4.8 ng/mL THC, 3.5 ng/mL 11-OH-THC, and 27 ng/mL THC-COOH.

CONCLUSIONS

The LC-MS/MS method developed in this study minimizes required sample volume and provides sensitive quantitation of THC, 11-OH-THC and THC-COOH in whole blood. Notably, our method simplifies sample preparation for analysis of THC and its metabolites in whole blood using an automated dispersive pipette extraction without subsequent dilution or solvent evaporation. The extraction process is rapid, minimizes matrix effects (~ 30% or less), and maximizes analyte recoveries (>93%). LODs and LOQs were below 0.75 ng/mL and 2 ng/mL, respectively. These outcomes clearly provide the necessary sensitivity to meet laboratory cut-offs with minimal imprecision (<8%). All calibrations were linear (R2 >0.99) over two orders of magnitude (0.5–50 ng/mL). This work was further expanded upon by the National Institute of Drug Abuse (NIDA) to include additional cannabinoid metabolites and application to urine (1-2).

REFERENCES

1. Andersson, M. (2016) Simultaneous quantification of 11 cannabinoids and metabolites in human urine by liquid chromatography tandem mass spectrometry using WAX-S tips. Analytical and Bioanalytical Chemistry 408(23): 6461-6471.
2. Scheidweiler, K. (2016) Quantification of cannabinoids and their free and glucuronide metabolites in whole blood by disposable pipette extraction and liquid chromatographytandem mass spectrometry. Journal of Chromatography A. 1453(1): 34-42.