K30 Quantification of Minor Blood Cannabinoids and Their Utility as Recent Cannabis Use Markers in Driving Under the Influence of Drugs (DUID) Investigation Cases

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After attending this presentation, attendees will be able to adopt and validate a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for quantitation of minor cannabinoids, including Cannabidiol (CBD), Cannabigerol (CBG), and Cannabinol (CBN). As CBG and CBN were reported as recent cannabis use markers, attendees will be able to apply the findings to improve the interpretation of recent cannabis use.

This presentation will impact the forensic science community by providing information on a quantitative method for minor cannabinoids as recent cannabis-use markers and their utility to aid in the interpretation of blood cannabinoid results in DUID cases.

It has been demonstrated that cannabinoid blood concentrations following smoking are not well correlated with effects because their concentrations are a function of various factors (dose, potency, route of administration, users' experience level, frequency of drug use, etc.), making interpretation of results challenging. Determination of the presence of minor cannabinoids in blood can add value to the interpretation by providing a time frame on last cannabis use.

This study describes a sensitive LC/MS/MS method for quantification of minor cannabinoids (CBD, CBG, and CBN). Cannabinoids were extracted from 100µL of whole blood using liquid-liquid extraction, separated in a two-dimensional LC system with an Agilent® Poroshell 120 PFP (4.6mm x 5mm; 2.7µm) as a guard column and an Agilent® Poroshell 120 Bonus RP (2.1mm x 50mm; 2.7µm) as an analytical column, in a 5min run time and detected by an AB SCIEX™ 6500 system with turbo ion spray operating in positive ion mode with scheduled mass spectrometric Multiple Reaction Monitoring (MRM). The method validation protocol was based on the Scientific Working Group for Forensic Toxicology (SWGTOX) guideline to include linearity, Limit Of Detection (LOD), Lower Limit Of Quantitation (LLOQ), precision and accuracy, interfering substances, extraction efficiency, matrix effect, stability, dilution of samples, matrix matching, and carryover.

The calibration for CBD, CBG, and CBN was linear from 0.1ng/mL to 50ng/mL. Minimum extraction efficiency and the maximum observed matrix effect were 97.4% and 2.8% suppression, respectively. The method also met validation criteria for precision and accuracy at the LLOQ, low and high controls, dilution of samples, matrix matching, interference, and carryover.

Between January and March 2017, NMS Labs received 2,787 cases for a basic DUID panel consisting of TEN common drugs of abuse. Cannabinoids were presumptively positive by Enzyme-Linked Immuno-Sorbent Assay (ELISA) in 52% (*n*=1,450) of cases and the presence of Δ^9-tetrahydrocannabinol (THC) was further confirmed in 1,202 cases (83%) by LC/MS/MS with a Reporting Limit (RL) 0.50ng/mL. Of those cases, 98 samples with positive THC at various concentrations were additionally tested for CBG, CBN and CBD using the method described. Table 1 summarizes these findings.

	CBG (ng/mL)	CBN (ng/mL)	CBD (ng/mL)
Mean (±SD) (ng/mL)	0.44 (±0.51)	0.28 (±0.15)	0.25 (±0.33)
Median (ng/mL)	0.26	0.23	0.12
Range (ng/mL)	0.10 – 3.3	0.10 – 0.74	0.10 – 1.1
% positive	74 (*n*=72)	67 (*n*=66)	10 (*n*=10)

Table 1. *Blood concentrations and positivity rates for CBG, CBN, and CBD*

To assess the correlation between concentrations of THC and three minor cannabinoids, the correlation coefficient (*r*) values were calculated and analyzed. The analysis demonstrated that both CBG and CBN have *r* values greater than Critical Values when *p*=0.05, suggesting a statistically significant positive correlation between THC and CBG as well as THC and CBN. The results from the same analysis between THC and CBD and CBG and CBN showed no correlation.

CBD had a significantly lower positivity rate compared to CBG and CBN. This, partly because of its short detection window and the various concentrations depending on the growth environment and strains, excluded CBD as a reliable recent cannabis use marker.

Using the incident and blood collection times provided in 42 cases with positive CBG and/or CBN at a RL of 0.1ng/mL, the calculated time difference (Δt) ranged from 0.033 to 5.4 hours. Of those cases, 11-hydroxy-THC (11-OH THC) was positive above RL of 1.0ng/mL in 39 cases (93%) with the mean and median concentrations of 5.1ng/mL and 3.9ng/mL, respectively (range: 1.4-22).

The detection windows were also evaluated for CBG and CBN at the previously studied RLs. Of 72 CBG-positive cases, a majority (>90%, *n*=65) were in between 0.1ng/mL and 1.0ng/mL; CBG was above 1.0ng/mL in seven cases with an average Δt of 1.1 hours (*n*=3; time information provided). Smoking higher doses of cannabis may explain the longer detection window for CBG in this study compared to the previously reported 0.5hr. Of 66 CBN-positive cases, six cases had CBN above 0.5ng/mL (RL 0.1ng/mL) with an average Δt of 0.78 hours (range; 0.68–1.13 hours, *n*=5; time information provided). This was consistent with the previous finding.

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