K41 Direct LC/MS/MS Quantification of Plasma Cannabinoids and Their Glucuronides

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After attending this presentation, attendees will be able to describe an LC/MS/MS method for the simultaneous identification and quantification of THC, its Phase I metabolites, 11-hydroxy-THC (11- OH-THC) and 11-nor-9-carboxy-THC (THCCOOH), other cannabis constituents, cannabidiol (CBD) and cannabinol (CBN), and its Phase II metabolites, THC-gluc and THCCOOH-gluc in 0.5 mL human plasma.

This presentation will impact the forensic science community by offering a novel analytical method for sensitive and specific

simultaneous quantification of both Phase I and II cannabinoid metabolites in a single plasma extract.

Introduction*:* Cannabis is the illicit substance most commonly detected in blood of driving under the influence of drugs (DUID) cases and in fatally injured drivers. Cannabinoid glucuronides have been proposed as potential markers of recent cannabis intake; however, to our knowledge, no method that directly detects and quantifies Δ^9- tetrahydrocannabinol (THC) and its metabolites THC-1-glucuronide (THC-gluc) and 11-nor-9-carboxy-THC glucuronide (THCCOOH-gluc) in plasma has been reported.

Method: Cannabinoids were extracted from 0.5 mL human plasma following pH adjustment with 1.5 mL 2% ammonium hydroxide (v/v), with reversed-phase polymeric SPE cartridges. Samples were reconstituted in 150 uL mobile phase consisting of 70% A (10 mM ammonium acetate, pH 6.15) and 30% B (acetonitrile). 30 uL was injected onto a LCMSMS instrument consisting of a Shimadzu SIL- 20ACXR auto-sampler, DGU-20A3 de-gasser, LC-20ADXR pumps, and CTO-20AC column oven interfaced with an Applied Biosystems 3200 Qtrap mass spectrometer equipped with a TurboV ion source operated in electrospray mode. Gradient chromatographic separation was achieved utilizing a Restek Ultra II Biphenyl HPLC column (100 x 2.1 mm, 3 µm particle size) with a 0.4 mL/min flow rate and an overall run time of 9 min. Detection and quantification were conducted in MRM mode with THC-gluc, THCCOOH-gluc, 11-OH-THC, THCCOOH and CBD
ionized in negative polarity mode while CBN and THC were ionized in positive polarity mode.

Results: Limits of quantification (LOQ) were 0.5 ng/mL for THC- gluc, 1.0 ng/mL for THC, CBD, CBN, 11-OH-THC and 5.0 ng/mL for THCCOOH and THCCOOH-gluc. Calibration curves were 0.5-50 ng/mL for THC-gluc, 1-100 ng/mL for THC, CBD, CBN, 11-OH-THC and 5.0-250 ng/mL for THCCOOH and THCCOOH-gluc (r^2 > 0.990 and concentrations ±15% of target, except at the LOQ where ±20% was acceptable). Validation parameters were tested at three concentrations spanning the linear dynamic range. Inter-day recovery (bias) and imprecision (*N*=18) were 100.0-108.0% of target concentration and 2.3- 8.0% relative standard deviation (RSD), respectively. Extraction efficiencies were 67.6 – 91.8%. Matrix effects ranged from -56.2 – 89.9%, depending on the analyte, with negative values indicating ion suppression; matrix effects at each quality control concentration were similar for native and corresponding deuterated compounds enabling low QC quantification within 88.1-117% of target concentration (*N*=18). Similar matrix effects were observed for twelve different blank plasma sources fortified with low quality control concentrations. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 24 h on the 4°C autosampler; losses of less than 17.9% were observed for each condition, except for THCCOOH-gluc that experienced losses up to 25.2% during storage for 24 h at room temperature. No quantifiable analyte carryover was observed at two times the upper LOQ.

Conclusions: A chromatographic method for the identification and quantification of cannabinoid metabolites in human plasma is described. This method will be employed in ongoing cannabinoid administration studies and will be useful for in assessing plasma cannabinoid concentrations in clinical toxicology and DUID cases.

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Cannabinoids, Glucuronides, LC/MS/MS

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