K2 An Analysis of Cannabinoids in Whole Blood

Adrian M. Taylor, PhD, SCIEX, 71 Four Valley Drive, Concord, ON L4K4V8, CANADA*

After attending this presentation, attendees will better understand how to effectively use protein precipitation and 2D Ultra High-Performance Liquid Chromatography/Mass Spectrometry (2D-UHPLC/MS) for the analysis of Δ^9-Tetrahydrocannabinol (THC), 11-OH-Δ^9-Tetrahydrocannabinol (THC-OH), and 11-nor-9-carboxy-Δ^9-Tetrahydrocannabinol (THC-COOH) in whole blood.

This presentation will impact the forensic science community by illustrating how combining a very simple sample preparation with a 2D-LC analytic approach in UHPLC allows for reaching the required sensitivity cut-offs with high-throughput capacity and robustness. This method claims to be a very interesting substitute to a classic technique based on Gas Chromatography/Mass Spectrometry (GC/MS) for the quantitation on cannabinoids in whole blood.

Hypothesis: Protein precipitation and 2D-UHPLC-MS provides effective clean-up of whole blood to analyze THC, THC-OH, and THC-COOH in a semi-automated way.

Methods: Sample Preparation – 100µL of whole blood (or calibrators, quality controls and unknowns) are mixed with 20µL of internal standard mixture in methanol (containing deuterated analytes). The protein precipitation step is done by the addition of 200µL of cold acetonitrile, 0.1% formic acid. After a ten minute incubation in the freezer, samples were centrifuged for five minutes at 13,000rpm. Next 200µL of supernatant were mixed with 200µL of water (0.1% formic acid) and were analyzed. The injection volume for each sample was defined at 100µL. **UHPLC Conditions –** Chromatographic separation was achieved using two Kinetex™ columns (PFP, 5µm and XB-C18, 5 µm). A Sciex 4500 QTRAP® was used for the MRM detection with an electrospray interface in positive/negative ionization mode. The run time of 2D-UHPLC program with equilibration was optimized at ten minutes using a solvent gradient of water 0.1% formic acid and methanol 0.1% formic acid.

Results: The sample preparation consists of a simple pre-treatment step using precipitation of whole blood samples, followed by dilution and injection. UHPLC methodology consists of a water/methanol mobile phase with an efficient 2D-LC gradient on Kinetex™ columns. Limits of quantitation were obtained below 0.5ng/mL for THC and its metabolites. Real samples were analyzed by this workflow. Results from a negative and a positive sample will be shown. Results are based on spiked standard curves in drug-free whole blood. The calculated concentrations for the positive sample were: 1.4ng/mL for THC, <0.5ng/ml for THC-OH, and 3ng/mL for THC-COOH.

Conclusion: Cannabinoids in whole blood analysis has been demonstrated on a 2D-UHPLC system, coupled to the SCIEX 4500 QTRAP® Mass spectrometer. Analysis is sensitive to detect concentration below 0.5ng/mL for each compound with Signal to Noise (S/N) greater than 10:1 (based on 3SD of the noise).

This method uses only 100µL of whole blood without offline solid phase extraction or liquid-liquid extraction. The short runtime allows for a high throughput whilst maintaining chromatographic performance batch after batch. No cross contamination was observed between the highest sample concentrations to the negative samples.

2D-UHPLC/MS, THC, Whole Blood