Stability of $\Delta^9$-Tetrahydrocannabinol (THC), 11-Hydroxy-$\Delta^9$-tetrahydrocannabinol (11-OH-THC), and 11-nor-$\Delta^9$-Tetrahydrocannabinol-9-carboxylic Acid (THCCOOH) in Whole Blood

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AIMS: Studies on cannabinoid stability in whole blood suggest that poor recovery of THC, 11-OH-THC and THCCOOH may result from binding to matrix components and container surfaces. Only one study has evaluated cannabinoid stability in whole blood stored in plastic tubes and none have reported changes to methodology attempting to improve recovery. Here, we report on cannabinoid recovery in whole blood samples stored in plastic at -20ºC, 4ºC and room temperature and methodological changes made in attempt to improve recovery from stored samples.

METHODS: Clinical study participants’ whole blood specimens are typically collected in Vacutainer® tubes containing anticoagulant, transferred to polypropylene cryotubes and stored at -20ºC until analysis. To approximate these conditions, blank whole blood pools (15 mL, NIH blood bank) were fortified at 0.35, 1, 2, 5, 10, 20, 30 or 60 ng/mL, aliquotted (1.2 mL), stored at -20ºC, 4ºC or room temp and analyzed in triplicate on days 1 (baseline), 3, 7 and 14. Calibrators (0.125 - 100 ng/mL) and quality control samples (0.35, 2, 20, 30, 60 and 90 ng/mL) were prepared by fortifying 1 mL whole blood with THC, 11-OH-THC and THCCOOH and d3 internal standards for each. Specimens were precipitated with 3 mL cold acetonitrile, extracted using Clean Screen® ZSTHC020 columns (United Chemical Technologies, Bristol, PA), and derivatized with BSTFA + 1% TMCS. Extracts were injected on an Agilent 6890 GC/5973MSD system (operated in EI/SIM mode). Additionally, changes to the precipitation and extraction were evaluated to determine whether recovery of analytes could be regained.

RESULTS: Two calibration curves (low, 0.125 - 25 and high, 25 - 100 ng/mL) were constructed with $r^2$ always > 0.99. Limits of quantification (LOQ) were 0.25 ng/mL for THC and THCCOOH and 0.5 ng/mL for 11-OH-THC. Intra- and inter-assay imprecision (%CV) was < 7% and < 9% respectively. Recovery of analytes was 85 - 104%. No chromatographic interference was detected from 20 over-the-counter, prescription and illicit drugs. After 14 d storage at -20ºC, percent recoveries (± SD) of THC, 11-OH-THC and THCCOOH ($n$=24), relative to baseline concentrations were 22.6 ± 13.2%, 46.7 ± 12.8% and 76.9 ± 9.9%, respectively. After 14 d storage at room temp, recoveries relative to baseline were 67.9 ± 9.0%, 69.9 ± 8.6% and 75.5 ± 5.8% ($n$=12). Recovery of cannabinoids from whole blood pools stored at 4ºC for 14 d was > 90% for all analytes. Methodological changes evaluated to improve analyte recovery were unsuccessful.

CONCLUSIONS: The analytical method reliably quantifies cannabinoids in freshly fortified whole blood. Data suggest reliable quantification of cannabinoids in freshly drawn authentic whole blood stored in polypropylene tubes for two weeks at 4ºC, however quantification may be low after storage at -20ºC due to significantly decreased analyte recovery.

Keywords: Cannabinoids, Recovery, Whole blood

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