K55 Whole Blood/Plasma Cannabinoid Ratios in Daily Cannabis Users After Multiple Years of Frozen Storage

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After this presentation, attendees will be informed of whole blood/plasma cannabinoid ratios in authentic specimens (N = 187 pairs) stored frozen for multiple years, and will learn of the first data on intra- subject whole blood/plasma ∆9-tetrahydrocannabinol (THC) and 11-nor-9- carboxy-THC (THCCOOH) ratio variability.

This presentation will impact the forensic science community by providing the first reference of cannabinoid whole blood/plasma ratios from aged authentic specimens and the first data on intra-subject whole blood/plasma THC and THCCOOH ratio variability.

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Thirty-two daily cannabis users (aged 19 - 38, 50% male, 84% African American) provided written informed consent for this IRB-approved study, and resided on a closed clinical research unit for seven days of monitored drug abstinence. Whole blood and plasma were collected simultaneously upon unit admission and every 24 h thereafter. Specimens were collected into Vacutainer tubes containing anticoagulant and transferred to polypropylene cryotubes for long term (-20ºC) storage. Cannabinoids were extracted by SPE (Clean Screen® ZSTHC020 extraction columns, United Chemical Technologies) and derivatized with BSTFA + 1% TMCS. Extracts were injected on an Agilent 6890 GC/5973MSD (operated in EI/SIM mode) retrofitted with a Dean's switch and cryotrap. Two calibration curves (low, 0.125 – 25 and high, 25 – 100 ng/mL) were constructed with $r^2 > 0.99$. Plasma limits of quantification (LOQ) were 0.25 ng/mL for THC and THC- COOH and 0.5 ng/mL for 11-OH-THC. Whole blood LOQ were 0.25 ng/mL for all analytes.

Overall mean ± SD whole blood/plasma ratios were 0.39 ± 0.17 (*N* = 75, median 0.39, range 0.08 – 0.77), 0.55 ± 0.22 (*N* = 17, median 0.56, range 0.22 – 0.90) and 0.45 ± 0.29 (*N* = 187, median 0.37, range 0.11 – 1.53) for THC, 11-OH-THC, and THCCOOH respectively. Mean whole blood/plasma ratios for THC and THCCOOH were determined for 13 subjects that had a least three paired specimens with THC and THCCOOH greater than LOQ (Table 1). A paired samples t test (α = 0.05) including only the 13 participants' paired THC and THCCOOH ratios revealed that the mean whole blood/plasma THC ratio was significantly lower than the correspon- ding mean THCCOOH ratio ($p < 0.01$). Four of 13 participants mean THCCOOH ratios (*N* = 7 each) were > 0.8. Removing these potential outliers yielded a non-significant difference ($p = 0.087$) between THC and THCCOOH mean ratios of 0.36 ± 0.10 and 0.46 ±, 0.13, respectively.

Table 1: Average of intra-subject mean (*N* = 3 – 7) whole blood/plasma THC and THCCOOH ratios from 13 participants over one week.

	N	Mean ± SD	Median	Range
THC	13	0.40 ± 0.11	0.40	0.13 – 0.56
THCCOOH	13	0.63 ± 0.28	0.56	0.28 – 1.15

These data impact the forensic community by providing the first reference of cannabinoid whole blood/plasma ratios from aged authentic specimens and the first data on intra-subject whole blood/plasma THC and THCCOOH ratio variability. The overall mean whole blood/plasma THC ratio was lower than previously reported, which may be explained by cannabinoid binding to whole blood proteins and/or container surfaces during storage. Mean intra-subject whole blood/plasma THC ratio was significantly lower than the corresponding mean THCCOOH ratio; however, further research may be necessary because data may be skewed by mean ratios >0.8 in four participants.

Cannabinoids, Ratio, Whole Blood

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