K40 In Vitro Free and Glucuronidated Cannabinoid Stability in Authentic Urine Following Controlled Smoked Cannabis

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After attending this presentation, attendees will be able to describe the phase I and II urinary cannabinoid stability in authentic urine stored for up to one year after controlled cannabis smoking under multiple storage conditions.

This presentation will impact the forensic science community by providing the most extensive cannabinoid stability data to date in authentic urine and aiding forensic toxicologists in cannabinoid test interpretation.

Analyte stability is an important factor in test interpretation. Phase I and II metabolite stability data in authentic urine specimens are limited. Only two studies documented 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) and THCCOOH-glucuronide stability in authentic urine. No studies evaluated Δ9-tetrahydrocannabinol glucuronide (THC-glucuronide) urinary stability. Phase I and II cannabinoid stability in authentic urine was evaluated for up to one year after controlled cannabis smoking under multiple storage conditions. This study hypothesized that cannabinoid stability would be affected in a time- and temperature-dependent manner.

Sixteen cannabis smokers (12M, 4F) provided written informed consent to participate in this Institutional Review Board- (IRB) approved study. For each participant, urine specimens collected between 0 and 6h after *ad libitum* cannabis smoking were combined in equal portions to form the high pool; low pool consisted of high pool diluted 1:5 (v/v) with drug-free urine. Pools were stored in polypropylene cryotubes in the dark and baseline concentrations were established by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) in triplicate within 24h at room temperature (RT), 4°C and -20°C. Stability was evaluated in duplicate samples after 1 week at RT; 1, 2, 4, 12, and 26 weeks at 4°C and -20°C; and 1 year at -20°C. Limits Of Quantification (LOQ) were: 2.0µg/L for THC and cannabinol; 1.0µg/L for THCCOOH, 11-hydroxy-THC (11-OH-THC), and cannabidiol; 0.5 µg/L for THC-glucuronide; and 5.0µg/L for THCCOOH-glucuronide. Stabilities at different storage times and temperatures compared to those at baseline were evaluated with Wilcoxon matched-pairs signed-ranks test (RT) or repeated-measures Friedman test (4°C and -20°C); Dunn's multiple comparisons tests were used for *post hoc* comparisons. Results with 2-tailed $P<0.05$ were considered significant.

At baseline, THCCOOH, THC-glucuronide, and THCCOOH-glucuronide were quantified in: 12, 16, and 15 low RT pools; 13, 16, and 15 high RT pools; 7, 16, and 15 low 4°C pools; 12, 16, and 15 high 4°C pools; 5, 15, and 15 low -20°C pools; and 12, 16, and 15 high -20°C pools, respectively. THC, cannabidiol, and cannabinol were never detected. RT THCCOOH baseline concentrations were significantly higher than at -20°C, but not 4°C. After one week at RT, THCCOOH increased, THCCOOH-glucuronide decreased, but THC-glucuronide remained unchanged. In RT low pool only, total THCCOOH (molar sum of THCCOOH and THCCOOH-glucuronide) was significantly lower after one week. At 4°C, THCCOOH was stable up to two weeks, THCCOOH-glucuronide up to one month, and THC-glucuronide for at least six months. At -20°C in both pools, THCCOOH was stable at one year, but high pool results at six months were high; THC-glucuronide and THCCOOH-glucuronide were stable for six months. Total THCCOOH was stable for at least six months at 4°C, and up to six months (low) and at least one year (high) at -20°C. Although not detected initially, 11-OH-THC was detected in two low and three high pools after one week at RT. These are the most extensive cannabinoid stability data to date in authentic urine.

Substantial THCCOOH-glucuronide deconjugation was observed at RT and 4°C and frozen specimen storage provided maximum stability, with cannabinoid glucuronides and total THCCOOH being stable for six months.

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Cannabinoids, Glucuronides, Stability

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