Evaluation of *E. coli* *β*-glucuronidase hydrolysis

To evaluate *E. coli* *β*-glucuronidase hydrolysis efficiency, THC blank plasma, urine and pH 6.8 phosphate buffer (five replicates each) were fortified with 75 μg/L THC-glucuronide and THCCOOH-glucuronide. Hydrolysis in urine and buffer were evaluated in addition to plasma because improved hydrolysis efficiency in these matrices over plasma would suggest possible hydrolysis interference from plasma components. Thirty samples from five different plasma pools also were prepared to determine differences in hydrolysis efficiency between individual pools. A fortified, but non-hydrolyzed sample was prepared to control for spontaneous conversion from glucuronidated to free cannabinoids. Co-extraction of glucuronides with free cannabinoids, and subsequent glucuronide hydrolysis during derivatization could yield artificially high free cannabinoid concentrations. To determine whether glucuronides were co-extracted with free cannabinoids, duplicate blank plasma samples fortified with 2, 20 and 200 μg/L THC- and THCCOOH-glucuronides were prepared. To determine glucuronide hydrolysis during derivatization, duplicate methanolic THC- and THCCOOH-glucuronides, free cannabinoid calibrators (0.25-25 μg/L) and free quality control samples (0.35, 2 and 20 μg/L) were prepared and derivatized without extraction. Total conjugated cannabinoids in authentic plasma may include sulfates and other conjugates that, when hydrolyzed using these procedures, could contribute to free cannabinoids. Potential sulfate conjugation was evaluated by hydrolyzing an authentic plasma pool of cannabinoids , for one, two, four or sixteen h, at 37 or 60°C, with 25 or 50 μL Glusulase® (Perkin-Elmer, Waltham, 90000 *β*–glucuronidase and 19000 sulfatase activity units/mL). We also evaluated relative hydrolysis efficiency in authentic plasma

after *E. coli* β–glucuronidase hydrolysis, tandem hydrolysis (β–glucuronidase followed by base hydrolysis using 2N sodium hydroxide) and base hydrolysis alone (duplicate specimens of each), as described by Abraham *et al*, 2007(1). The procedures that produced maximum hydrolysis, with acceptable chromatography for all analytes were adopted.

References

1. Abraham TT, Lowe RH, Pirnay SO, Darwin WD, Huestis MA. Simultaneous GC-EI-MS determination of delta9-tetrahydrocannabinol, 11-hydroxy-delta9-tetrahydrocannabinol, and 11-nor-9-carboxy-delta9-tetrahydrocannabinol in human urine following tandem enzyme-alkaline hydrolysis. J Anal Toxicol 2007;31:477-85.