Analysis of Cannabinoids by Isomer-Selective UPLC-MS-MS Analysis in Urine for Clinical Research

Thomas G. Rosano* [1,2], Jane A. Cooper [3], Kiley L. Scholz [1], Jonathan Danaceau [4] and Michelle Wood [1]

1 National Toxicology Center, Center for Medical Science, Albany NY, USA
2 Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, New York, USA
3 Forensic Testing Services, Mirfield, UK
4 Waters Corporation, Milford, MA, USA

Background: Confirmation of cannabinoid use in urine has traditionally focused on Δ9-tetrahydrocannabinol (Δ9-THC) with analysis of its major metabolite, 11-nor-9-carboxy-Δ9–tetrahydrocannabinol (Δ9-cTHC) in free and conjugated forms. Legalization of hemp, however, has led to widespread production and sale of cannabidiol (CBD) derivatives with psycho-activity, including Δ8-THC and Δ10-THC isomers. The increasing availability and growing use of isomer derivatives necessitates an expanded scope of cannabinoid methods.

We report a clinical research method for quantitative, isomer-selective method of cannabinoid confirmation by liquid chromatography/tandem mass spectrometry (UPLC-MS-MS) for determination of parent-drug isomers (Δ8-THC, Δ9-THC, Δ10-THC, CBD), as well as isomeric metabolites (Δ8-cTHC, Δ9-cTHC).

Methods: Optimum resolution with minimum analytical run time was achieved by employing a high efficiency solid-core particle column (CORTECS UPLC C18+, 1.6µm, 2.1 x 50 mm) eluted with a mixture of 0.1% formic acid in water and acetonitrile. A rapid method of hydrolysis, dilution, and UPLC-MS-MS analysis was employed for quantitative co-determination of free and conjugated analytes in 220 samples, using stable isotope internal standardization.

Results: Acceptable performance was achieved for the evaluated method characteristics which included: precision (low and high QCs were within 14.5%), accuracy (within 11.5% bias), carryover, dilution linearity demonstrated for 10-fold pre-analysis dilution, matrix effects and interference. A 14% prevalence for the Δ8-cTHC isomer was determined in samples. CBD prevalence was 10% based on the parent drug testing. Majority of samples (99%) were positive for Δ9-cTHC (average 766 ng/mL, maximum 8880 ng/mL), and Δ8-THC, Δ9-THC, and Δ10-THC, were not detected in any samples. A comparison of Δ8-cTHC and Δ9-cTHC phase two metabolism is also reported and demonstrates parallel excretion-kinetics for the isomer metabolites.

Conclusion: A simple, rapid and selective clinical toxicology research method for Δ8-THC, Δ9-THC, Δ10-THC, CBD, Δ8-cTHC and Δ9-cTHC in urine was developed. Application studies showed prevalence of Δ8-cTHC isomer in samples and revealed a significant concomitant use of Δ8-THC and Δ9-THC.

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Keywords: UPLC-MS-MS; urine cannabinoid isomer confirmation; Δ8-tetrahydrocannabinol; Δ9-tetrahydrocannabinol; Δ10-tetrahydrocannabinol; cannabidiol