Validated method for the determination of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC in oral fluid, urine and blood using solid-phase extraction and liquid chromatography- mass spectrometry with electrospray ionization

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AIMS: A fully validated, sensitive and specific method for the extraction and quantification of Δ^9-Tetrahydrocannabinol (THC), 11-Hydroxy-Δ^9-THC (11-OH-THC) and 11-nor-9-carboxy-Δ^9-THC (THC-COOH) in oral fluid, urine and whole blood is presented. These results will be used to analyse oral fluid, urine and blood samples collected from volunteers participating in a controlled Cannabis smoking administration study, with a previous defined protocol of samples collection.

METHODS: The method comprised two different solid-phase extraction procedures, with Bond Elut LRC-Certifiy columns (10cc, 300mg), for oral fluid and urine samples and Clean Screen ZSTHC020 for blood samples, followed by Liquid Chromatography-Mass Spectrometry analysis on a 2695 Alliance System and a ZQ 2000 Mass Spectrometer from Waters, using positive and negative–mode electrospray ionization in the selected ion-recording (SIR) mode. Chromatographic separation was achieved using a Symmetry® C18 column (2.1x150mm, 5µm), eluted isocratically with acetonitrile: formic acid 0.1% (70:30, v/v), at a 0.3 ml/min flow rate. Quantitation was achieved by the addition of two deuterated analogues as internal standards, THC-d_3 and THC-COOH-d_3. The compounds were quantified by selected ion-recording of m/z 315.31 (confirmation ions: 193.13 and 259.14), 329.18 (confirmation ion: 311.20), 343.16 (confirmation ions: 299.17 and 365.19) for THC, 11-OH-THC and THC-COOH, respectively, and m/z 318.27 and 346.26 for the deuterated internal standards, THC-d_3 and THC-COOH-d_3, respectively. The results of only the quantifier ion are presented although three ions were monitored for THC and THC-COOH and two for 11-OH-THC.

RESULTS: No interferences were detected in 10 blank oral fluid, urine and blood samples. Intra-day and inter-day coefficients of variation values were determined by replicate analyses (n=5) of oral fluid, urine and blood aliquots. Three concentration levels were selected for validation (25, 100 and 1000 ng/mL). The method proved to be precise for Δ^9-THC and Δ^9-THC-COOH both in terms of intra-day and inter-day analysis, with intra-day coefficients of variation (CV) less than 6.5, 6.5 and 6.2% for THC in saliva, urine and blood respectively and 6.8 and 7.7% for THC-COOH in urine and blood respectively. Day-to-day CVs were less than 3.5, 4.9 and 11.3% for THC in saliva, urine and blood respectively and 6.2 and 6.4% for THC-COOH in urine and blood respectively. Accuracy was 83±8%, 83±6%, 86±4% for THC in saliva, urine and blood respectively and 85±4% and 83±9% for THC-COOH in urine and blood respectively. In oral fluid, the calculated extraction efficiencies for Δ^9-THC ranged from 73 to 90%; in urine,

calculated extraction efficiencies for Δ9-THC ranged from 75 to 86%, for Δ9-THC-OH ranged from 37 to 39%, and for Δ^9-THC-COOH ranged from 80 to 87%. In blood the calculated extraction efficiencies for Δ^9-THC ranged from 79 to 85%, for $\Delta^9$9-THC-OH from 60 to 93%, and for Δ^9-THC-COOH from 77 to 92%. Limits of detection (LOD) where 2 ng/mL for THC in oral fluid, 0.5, 25 and 0.5 for THC, 11-OH-THC and THC-COOH, respectively in urine and blood. Two different calibration curves were used, for high and low concentrations, with linearity from 2 to 300 ng/mL and 300 to 2000 ng/mL for THC and THC-COOH and from 25 to 300 ng/mL and 300 ng/mL to 2000 ng/mL for THC-OH.

CONCLUSIONS: The procedure presented here has high specificity, selectivity and sensitivity. It can be regarded as an alternative method to GC/MS for the confirmation of positive immunoassay test results, and can be used as a suitable analytical tool for the identification of THC and its metabolites in oral fluid, urine and blood samples. In contrast to existing GC-MS methods, no time-consuming derivatisation or silanisation steps were needed. However, the method was not precise for THC-OH, being only useful for its identification, detection and confirmation.

KEYWORDS: *Cannabinoids, Oral fluid, Urine, Blood, LC-MS*

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