Analysis of THC, 11-OH-THC, THC-COOH, CBD and CBN by Liquid Chromatography-tandem Mass Spectrometry in human EDTA-plasma and urine after small doses of THC and Cannabis Sativa extract

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Besides the psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC), hashish and marijuana as well as *Cannabis sativa* extracts contain varying amounts of cannabidiol (CBD) and of the degradation product cannabinol (CBN). The additional determination of these compounds is interesting from a toxicological and medical point of view because it can be used for further proof of cannabis exposure due to of pharmacodynamic interaction of CBD on Δ^9-THC.

We performed randomized, single center, three-periods cross-over clinical study,, where five different capsule formulations of *Cannabis sativa* extract and a synthetic Δ^9-THC compound of 20 mg Δ^9-THC_{tot} were administered.

A method for the simultaneous quantitative determination of δ-THC, its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ^9-tetrahydro-cannabinol (THC-COOH), CBD and CBN from human EDTA-plasma and urine was developed and validated. The method is based on solid-phase extraction for human EDTA-plasma and liquid-extraction for human urine. Liquid chromatography-tandem mass spectrometry (Thermo Finnigan LCQ Advantage) was performed on a Synergi MAX-RP 80A C_{12} column and equipped with an APCI method operating in the positive detection mode. Linearity was assessed with calibration curves ranging from 1 to 100 ng/ml in bovine serum and human urine. Quantification was performed by % integration of the area under the specific peaks in relative to the area of external and internal standards (IS: Δ^9-THC-D3, THC-COOH-D3).

The limit of quantification in EDTA-plasma was 3 ng/ml (CBN, Δ^9-THC, 11-OH-THC) and 2 ng/ml (THC-COOH, CBD), in urine 4 ng/ml (CBN), 1 ng/ml (CBD, Δ^9-THC), 3 ng/ml (THC-COOH) and 5 ng/ml (11-OH-THC).

The substance stability tests showed the cannabinoids to be stable at -70°C in both matrices for 20 weeks in silanized glass vials. Degradation of THC-COOH-glucuronide followed an apparent first-order process that led to the formation of THC-COOH.

One test person showed the following EDTA-plasma concentrations of Δ^9-THC after intake of a capsule formulation of a *Cannabis sativa* extract: 0h: 0 ng/ml, 0.5h: 3.208 ng/ml, 1h: 4.614 ng/ml, 2h: 4.717 ng/ml, 4h: 3.219 ng/ml, 8h: 2.871 ng/ml, 12h: 2.865 ng/ml, 24h: 2.853 ng/ml.

Interassay precision was established by measuring with commercially available quality controls (BioRad, Liquichek™ urine toxicology controls). Eight samples per concentration weby re measured.determination of eight samples per concentration. The first target value was 37.5 ng/ml, mean was 27.9 ng/ml, CV was 15.5 %. The second target value was 65.0 ng/ml, mean was 64.7 ng/ml, CV was 6.5%. The third target value was 125.0 ng/ml, mean was 132.6 ng/ml, CV was 2.9%.

CONCLUSIONS: The limits of quantification and detection, the linear measuring range, the stability of the substances in blood plasma and urine and the good inter and intra assay precision The validation of the method showed a good satisfying overall analytical performance of the method, well suited for clinical applications..

KEYWORDS: *Delta-9-THC, Cannabis sativa extract, Metabolites, Liquid chromatography-tandem mass spectrometry, Validation, EDTA-plasma, Urine, Solid-phase extraction, Liquid-phase extraction, Precision, Stability, Clinical study*

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