

S1 Quantitation of THC and its metabolites (11OH-THC, THC-COOH) by GC–MS

Water, methanol (MeOH), acetonitrile (ACN) and acetone (all HPLC grade) were purchased from Honeywell (Seelze, Germany). Acetic acid (purissima) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Reference standards of Δ^9 -tetrahydrocannabinol (THC), THC- d_3 , 11-hydroxy- Δ^9 -tetrahydrocannabinol (11-OH-THC), 11-OH-THC- d_3 , 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH), and THC-COOH- d_3 were obtained from Lipomed (Arlesheim, Switzerland).

Blank samples spiked with known concentrations of the reference compounds served as calibration standards and deuterated analogues of the analytes were used as internal standards.

One milliliter of sample was mixed with 20 μ L internal standard solution (0.4 μ g THC- d_3 , 0.4 μ g 11-OH-THC- d_3 , 1.0 μ g THC-COOH- d_3 per mL MeOH) and 2 mL acetonitrile for protein precipitation. The sample was vortexed for 20 seconds and centrifuged for 5 minutes at 4000 x g. The supernatant was separated, spiked with 5 mL 0.1 M acetic acid solution, and submitted to solid-phase extraction employing Chromabond Drug II columns (200 mg/3 mL, Macherey-Nagel, Dueren, Germany). The cartridges were equilibrated with 2 mL methanol and 2 mL water. After sample application, the cartridges were washed twice with 3 mL water and dried for 20 minutes. Two 1-mL aliquots of an acetic acid solution in acetone (1%, v/v) were used for elution. The eluate was evaporated to dryness and reconstituted in 0.3 mL dimethyl sulfoxide/tetramethylammonium hydroxide (both Sigma-Aldrich). The solution was incubated for 2 minutes prior to the addition of 50 μ L iodomethane (Sigma-Aldrich). The reaction mixture was kept at room temperature for 15 minutes before the addition of 700 μ L 0.1 M HCl solution (Sigma-Aldrich). The subsequent liquid-liquid extraction was accomplished by adding twice 2 mL hexane (Sigma-Aldrich). The collected organic layers were evaporated to dryness and reconstituted in 50 μ L heptane (Sigma-Aldrich). This final extract was submitted to GC–MS analysis.

The GC–MS system consisted of a 6890N GC device with a 5973 inert mass-selective detector (Agilent Technologies, Santa Clara, CA, USA). A 30 m $\times$ 0.25 mm, 0.25- μ m DB- XLB column from J&W Scientific (Agilent) was used for chromatographic separation. Carrier gas was helium with a flow rate of 1.0 mL/min. The injection volume was 1.0 μ L (splitless). The injection temperature was 250°C. The temperature program was as follows: 50°C, hold 1 min; increase to 150°C with 25°C/min, to 320°C with 10°C/min, hold for 8 min and to 330°C in 20°C/min, hold for 7.5 min. MS was performed in electron ionization mode (70 eV). Mass spectral data were recorded on a personal computer with the HP MS ChemStation software G1034C version D01.00 (Agilent Technologies). The following ions were targeted in selected ion monitoring mode: 328, 285, 245 (THC ME), 331, 288, 248 (THC- d_3 ME), 313, 358, 231 (11OH-THC 2 ME), 316, 361, 234 (11OH-THC- d_3 2 ME), 372, 357, 313 (THC-COOH 2ME) and 375, 360, 316 (THC-COOH- d_3 2 ME).

The method was validated according to guidelines published by the Society of Toxicological and Forensic Chemistry (1). The calibration ranges were 1–10 µg/L for THC and 11-OH-THC, as well as 3.75–50 µg/L for THC-COOH. The limits of detection were 0.5 µg/L for THC and 11-OH-THC and 2.0 µg/L for THC-COOH, respectively. Bias and relative standard deviations were <15%.

Reference

1. Peters, F.T., Hartung, M., Herbold, M., Schmitt, G., Daldrup, T., Mußhoff, F. (2009) APPENDIX B To the GTFCh Guidelines for quality assurance in forensic-toxicological analyses: Requirements for the validation of analytical methods. *Toxichem Krimtech*, **76**, 185-208.

S2 LC–MS-MS analysis of different forms of hydroxylated hexahydrocannabinol as well as 11-nor-9(R)-carboxy-hexahydrocannabinol

MilliQ Water was generated from in-house water system ($\geq 18.2 \text{ M}\Omega\cdot\text{cm}$), acetonitrile (ACN) and formic acid (all HPLC grade) were purchased from J.T Baker and Millipore Sigma respectively. 10(R)-hydroxy-9(S)-hexahydrocannabinol, 11-hydroxy-9(S)-hexahydrocannabinol, 9 β -hydroxy-hexahydrocannabinol, 8(S)-hydroxy-9(R)-hexahydrocannabinol, and 11-nor-9(R)-carboxy-hexahydrocannabinol were obtained from Cayman Chemical Company (Ann Arbor, MI, USA).

The LC–MS-MS system consisted of a Sciex ExionLC device coupled to a TripleQuad 4500 QTRAP+ mass spectrometer (Sciex, Toronto, Canada). The chromatographic separation was accomplished on an Ascentis Express C18 column ($2.7 \mu\text{m}$, $90 \text{ \AA}$, $100 \times 2.1 \text{ mm}$; Merck Supelco, Darmstadt, Germany) using a 3-minute linear gradient of 50–70% ACN in an aqueous 0.1% formic acid solution. The column temperature was 40°C . The flow rate was $500 \mu\text{L}/\text{min}$. The injection volume was $1.0 \mu\text{L}$. The mass spectrometer was operated in positive electrospray ionisation (ESI+) mode. The spray voltage was set to 4.5 kV . Gas flows were 60 arbitrary units for the nebulizer gas and 50 arbitrary units for the turbo gas. The temperature of the turbo gas was adjusted to 550°C . For acquiring MS-MS spectra, the precursor ion scan mode (either at m/z 333 or m/z 347) was used. The scan range was m/z 50–350. The collision energy ranged from 5–60 eV.

Figure S1. Fragmentation spectra of different hydroxylated forms of hexahydrocannabinol.

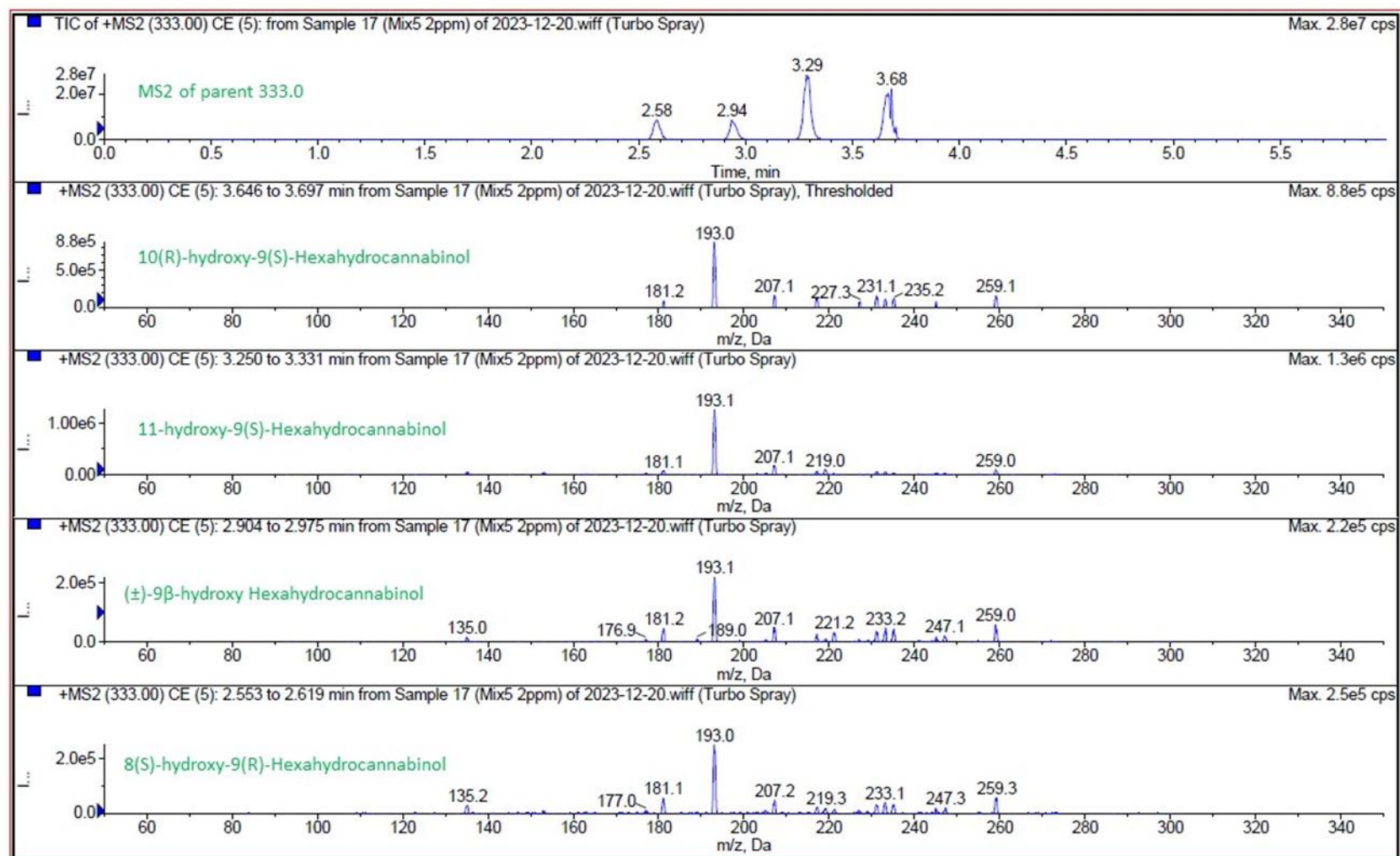


Figure S2. Fragmentation spectrum of 11-nor-9(R)-carboxy-hexahydrocannabinol.

