Determination of Δ^9-Tetrahydrocannabinolic Acid A in Serum Samples by GC-MS

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Abstract

The aim of this work was the extension of our standard procedure for quantitation of Δ9-tetrahydrocannabinol (Δ9-THC) and its main metabolites by GC-MS for the simultaneous determination of Δ9-tetrahydrocannabinolic acid A (Δ9-THCA A), the non-psychoactive precursor of Δ9-THC. The sample preparation consisted of a solid-phase extraction procedure (Chromabond C_{18}, 3 mL, 500 mg) and subsequent derivatization with N-methyl-N-(trimethylsilyl-trifluroracetamide (MSTFA). The extracts were analyzed using a GC-MS system (Agilent 6890 GC system, Agilent 5973 MSD) equipped with a DB-5 capillary column (0.25 mm x 30.0 m, I.D. 250 nm). For quantitation, the mass selective detector was operated in the SIM mode and for the determination of Δ9-THCA A, an additional time window was created with the m/z values of the TMS-derivative of Δ9-THCA A (m/z 487, m/z 488, m/z 489). A six-point calibration with 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-D_3 as internal standard was used for quantitation of Δ9-THCA A. Good linearity was achieved in the concentration range from 1 to 7.5 ng/mL (r^2 = 0.9933). The limit of detection was 0.6 ng/mL and the limit of quantitation was 2.0 ng/mL. To date, 148 blood samples have been analysed. Δ9-THCA A could be detected in levels higher than the LOD in 87 % of 137 Δ9-THC positive blood samples. However, LOQ was only exceeded in 17 cases (12 %).

1. Introduction

Δ9-THCA A is a non-psychoactive precursor of Δ9-THC, the main psychoactive component of marihuana and hashish. In hemp plants, the biogenesis of Δ9-THCA A, an aromatic polyketide substituted with a terpene moiety, starts with olivetolic acid, which is formed from activated acetic acid as starter molecule and five mevalonate units. Olivetolic acid is then alkylated with activated geraniole and yields cannabigerolic acid [3, 6]. From this compound, Δ9-THCA A is formed via the intermediate product cannabinolic acid.

In fresh plant material about 90 % of the total Δ9-THC is available as Δ9-THCA A, during storage and fermentation degradation to Δ9-THC occurs [1, 5]. When heated or smoked, Δ9-THC is partially released from Δ9-THCA A by decarboxylation. A recently published study investigated the thermal conversion of Δ9-THCA A to Δ9-THC under different analytical conditions and under smoking conditions. Under optimized analytical conditions, i.e. temperatures

higher than 140°C prior to analysis, about 70 % of Δ9-THCA A are converted to Δ9-THC, while in the simulation of the smoking process, only about 30 % of the spiked Δ9-THCA A could be recovered as Δ9-THC and thermal decomposition by combustion has to be considered as the reason for losses of up to 70 % of Δ9-THCA A [2].

As the release of Δ9-THC from its precursor Δ9-THCA A during smoking is incomplete, Δ9-THCA A can be detected in serum and urine of cannabis consumers [4]. In practical forensic-toxicological analysis, the determination of Δ9-THCA A additionally to Δ9-THC and its main metabolites 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-Δ9-THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (Δ9-THC-COOH) in serum samples of drivers suspected for driving under the influence of drugs (DUID) has not been considered as marker for cannabis consumption due to its assumed complete thermal conversion during smoking. Several procedures have been published for the determination of Δ9-THC and its metabolites by GC-MS in body fluids, but none of these assays covered Δ9-THCA A. Therefore we decided to include Δ9-THCA A into our standard procedure for quantitation of Δ9-THC, 11-OH-Δ9-THC and Δ9-THC-COOH by GC-MS for the simultaneous determination of Δ9-THCA A as well as of Δ9-THC and its metabolites during routine analysis of serum samples from cases suspected for DUID.

2. Experimental

2.1 Chemicals and Materials

Δ9-THCA A reference standard was obtained from Lipomed (Bad Säckingen, Germany). The methanolic solutions (1 mg/mL) of Δ9-tetrahydrocannabinol-D_3 (Δ9-THC-D_3), 11-hydroxy-Δ9-tetrahydrocannabinol-D_3 (11-OH-THC-D_3), and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-D_3 (Δ9-THC-COOH-D_3) were purchased from Promochem (Wesel, Germany). N-methyl-N-(trimethylsilyl)-trifluoracetamide (MSTFA) was obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). Solid-phase extraction (SPE) columns (Chromabond C_{18}, 3 mL, 500 mg) were supplied by Macherey-Nagel (Düren, Germany).

2.2 Serum Samples

Authentic serum samples were collected from drivers suspected for DUID and submitted to our laboratory for toxicological analysis. Pooled blank serum samples were used for quantitation and were obtained from healthy drug-free volunteers. All samples were stored at -20°C prior to analysis.

2.3 Sample Preparation

25 µL of a methanolic internal standard (IS) solution containing 5 ng Δ9-THC-D_3, 5 ng 11-OH-THC-D_3, and 25 ng Δ9-THC-COOH-D_3 were added to the serum samples (1 mL). After dilution with 2 mL 0.1 M acetic acid, the samples were shortly mixed and loaded on SPE columns, which had been preconditioned with 2 mL methanol and 2 mL 0.1 M acetic acid each at a flow rate of 2 mL/min. The samples were rinsed through the sorbent bed at a flow rate of 1 mL/min and the SPE columns were washed with 1 mL 0.1 M acetic acid and acetonitrile/water (70:30, v:v) each at a flow rate of 1 mL/min. Reduced pressure was applied for 10 min. Elution was performed with 1.5 mL acetonitrile at a flow rate of 1 mL/min. The extracts were evaporated to dryness at 60°C under a stream of nitrogen. After addition of 25 µL ethyl acetate and of 25 µL MSTFA, the reaction vials were sealed and shortly mixed. Derivatization was carried out in the heating block at 90°C for 45 min. 1 µL of the derivatization mixture was injected into the GC-MS system.

2.4 Gas chromatography-mass spectrometry

The samples were analyzed using an Agilent 6890 Series GC system (Waldbronn, Germany) combined with a CTC analytics CombiPal (Zwingen, Switzerland), an Agilent 5973 series mass selective detector (Waldbronn, Germany) and Chem Station G1701DA version D.00.00.38.

The GC conditions were as follows: splitless injection mode; column, Optima-5-MS capillary (30 m x 0.25 mm I.D. x 0.25 µm film thickness) Macherey-Nagel, Düren, Germany), injection port temperature, 250°C; carrier gas, helium; flow rate, 1.0 mL/min; oven temperature, 140°C for 3 min, increased to 200°C at 30°C/min, to 210°C at 5°C/min, to 240°C at 2°C/min, to 310°C at 30°C/min, and was held at this temperature for 4 min.

The MS conditions were as follows: transfer line heater, 280°C; source temperature, 230°C; electron impact ionization (EI) mode; ionization energy, 70 eV; selected ion monitoring (SIM) with the following program: solvent delay, 10 min; 10.00-17.00 min, *m/z* 306, 374, 389 (target ion, t) for Δ9-THC-D_3 and *m/z* 303, 371, 386 (t) for Δ9-THC; 17.00-22.90 min, *m/z* 374 (t), 462, 477 for 11-OH-Δ9-THC-D_3 and *m/z* 371 (t), 459, 474 for 11-OH-Δ9-THC; 22.90-23.50 min, *m/z* 374 (t), 476, 491 for Δ9-THC-COOH-D_3 and *m/z* 371 (t), 473, 488 for Δ9-THC-COOH. The electron multiplier voltage (EMV) offset was set at 200 V.

3. Results and Discussion

3.1 Sample Preparation

The analytes were isolated from one milliliter of serum using an SPE based procedure, previously proven to be appropriate for the extraction of Δ9-THC and its metabolites. This sample volume was necessary to allow the reliable quantitation of the analytes at low concentrations.

3.2 Gas chromatography-mass spectrometry

The analytes were separated by GC and detected by MS operated in the SIM mode. Three characteristic ions (one target and two qualifier ions) were monitored for each analyte and IS. For the determination of Δ9-THCA A, an additional time window was created with the m/z values of the TMS-derivative of Δ9-THCA A (m/z 487, 488, 489), which were chosen from the corresponding full-scan mass spectrum. Only one intensive fragment ion (m/z 487) was detected in the spectrum of Δ9-THCA A. The fragment ions m/z 488 and m/z 489 are isotope peaks of the base peak m/z 487. Furthermore, two additional characteristic ions (m/z 502, m/z 339) occurred in the spectrum, but their intensity was insufficient for use as qualifier ions. Without other suitable fragment ions, the base peak and its two isotope peaks were chosen for SIM analysis.

The method showed high selectivity. In ten different blank serum samples no peaks interfering with Δ9-THCA A were observed. Quantification was carried out using calibration curves obtained with spiked calibrators. For Δ9-THCA A, a six-point calibration with Δ9-THC-COOH-D_3 as IS was used. With an unweighted linear regression model good linearity (r^2=0.9933) was achieved over a concentration range from 1 to 7.5 ng/mL Δ9-THCA A. The limits of detection (LOD) and quantification (LOQ) were calculated using the software B.E.N.® resulting in 0.6 ng/mL for the LOD and 2.0 ng/mL for the LOQ. Two quality control samples (QC) were analyzed in each run. Inter-day repeatability as tested at 1 ng/mL (low QC sample) and at 5 ng/mL (high QC sample): relative standard deviations were 4.55 % for the low concentration level and 9.34 % for the high concentration level, respectively.

To date, 148 serum samples have been analyzed with the extended GC-MS procedure. Cannabis consumption has been confirmed in 137 cases. In 119, i.e. 87 % of these Δ9-THC positive cases, Δ9-THCA A was detected in levels higher than the LOD. However, the LOQ of 2 ng/mL was only exceeded in 17 cases (12 %). Altogether, the calculated Δ9-THCA A concentrations ranged from 0.6 to 9.8 ng/mL. In Fig. 1, merged mass fragmentograms of target and qualifier ions of the analytes from a real sample are shown.

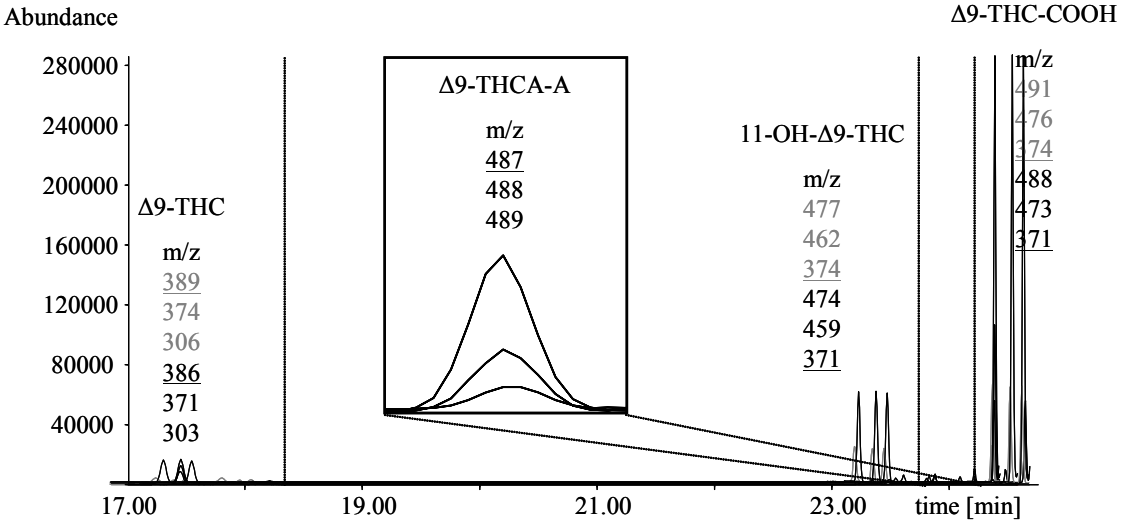

Figure 1. Merged mass fragmentograms with the given ions of an authentic serum sample after SPE. The dotted lines separate the SIM time windows. The peaks correspond to 23 ng/mL Δ9-THC, 10 ng/mL 11-OH-Δ9-THC, 4.1 ng/mL Δ9-THCA A and 88 ng/mL Δ9-THC-COOH.

Furthermore, correlations between the calculated Δ9-THCA A concentrations and the respective concentrations of Δ9-THC and its metabolites were studied. As expected, no correlations between these levels were observed due to variable Δ9-THCA A contents in the material consumed and different conditions and techniques of consumption.

4. Conclusions

The GC-MS assay presented here allows the simultaneous quantitation of Δ9-THCA A, Δ9-THC, and its metabolites 11-OH-Δ9-THC and Δ9-THC-COOH and thus the determination of Δ9-THCA A during routine analysis of serum samples from cases suspected for DUID. The results of 137 analyzed cases show, that more than 80% of the Δ9-THC-positive samples were also positive for Δ9-THCA A. Therefore Δ9-THCA A represents a promising marker for Δ9-THC abuse, but for future work lower limits of quantitation are preferable. For the interpretation of Δ9-THCA A concentrations in relation to the concentrations of Δ9-THC and Δ9-THC metabolites information on the elimination kinetics of Δ9-THCA A are required. Furthermore, studies on the metabolism of Δ9-THCA A are planned. Ideally, conclusions about the kind of material consumed, the way of application and/or the time of intake could be drawn by synopsis of the concentrations.

5. References

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