Simultaneous and Sensitive Analysis of THC, 11-OH-THC, THC-COOH, CBD, and CBN by GC–MS in Plasma after Oral Application of Small Doses of THC and Cannabis Extract

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Abstract

Besides the psychoactive Δ^9-tetrahydrocannabinol (THC), hashish and marijuana as well as cannabis-based medicine extracts contain varying amounts of cannabidiol (CBD) and of the degradation product cannabinol (CBN). The additional determination of these compounds is interesting from forensic and medical points of view because it can be used for further proof of cannabis exposure and because CBD is known to modify the effects of THC. Therefore, a method for the simultaneous quantitative determination of THC, its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), CBD and CBN from plasma was developed. The method was based on automatic solid-phase extraction with C_{18} ec columns, derivatization with *N,O*-bistrimethylsilyltrifluoroacetamide (BSTFA), and gas chromatography–electron impact ionization-mass spectrometry (GC–EI-MS) with deuterated standards. The limits of detection were between 0.15 and 0.29 ng/mL for THC, 11-OH-THC, THC-COOH, and CBD and 1.1 ng/mL for CBN. The method was applied in a prospective pharmacokinetic study after single oral administration of 10 mg THC alone or together with 5.4 mg CBD in cannabis extract. The maximum plasma concentrations after cannabis extract administration ranged between 1.2 and 10.3 ng/mL (mean 4.05 ng/mL) for THC, 1.8 and 12.3 ng/mL (mean 4.9 ng/mL) for 11-OH-THC, 19 and 71 ng/mL (mean 35 ng/mL) for THC-COOH, and 0.2 and 2.6 ng/mL (mean 0.95 ng/mg) for CBD. The peak concentrations (mean values) of THC, 11-OH-THC, THC-COOH, and CBD were observed at 56, 82, 115, and 60 min, respectively, after intake. CBN was not detected. Caused by the strong first-pass metabolism, the concentrations of the metabolites were increased during the first hours after drug administration when compared to literature data for smoking. Therefore, the concentration ratio 11-OH-THC/THC was discussed as a criterion for distinguishing oral from inhalative cannabis consumption.

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Introduction

There is an increasing interest in analytical data of cannabinoids in human samples from a forensic as well as from a clinical point of view. The growing use of this drug and the enduring discussion about its legalization in several countries require more information about its pharmacokinetic behaviour as a prerequisite for the development of traffic safety standards and for evaluation of concentrations in traffic cases and other offences (1). In the same way, more data about concentration/effect relationships are needed in context with the introduction of Δ^9-tetrahydrocannabinol (THC) as a remedy with anti-emetic, appetite-inducing, muscle-relaxant, analgesic, antiglaucoma, anti-asthmatic, and anti-epileptic indications (2).

The pharmacokinetics of cannabinoids were recently reviewed by Grotenhermen (3). The course of the plasma concentrations was measured after smoking (4–14), oral intake (4,14–18), and intravenous injection (4,7,8,14–16,18,19) of THC or cannabis products by several authors. In most studies, the analysis is limited to THC and its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH). However, after consumption of marijuana or hashish, the main constituents of cannabis cannabidiol (CBD) and cannabinol (CBN) should also be considered.

After THC, CBD is the most abundant cannabinoid in cannabis plants. According to newer results (20,21), the carboxylated precursors in the biosynthesis of CBD and THC are both directly derived from cannabigerolic acid by different enzymes. The existence of the postulated enzyme CBD-cyclase catalyzing the synthesis of THC via CBD has not been experimentally confirmed. The CBD/THC ratio is mainly dependent on the genetic background of the individual plant. With respect to the cannabinoid composition, the cannabis

plants can be divided into a chemotype with a high THC and a low CBD content, an intermediate chemotype with a prevalent CBD content and THC present at various concentrations, and a chemotype with a particularly low THC content. The average concentrations in more than 30,000 marijuana preparations confiscated in the U.S. between 1980 and 1997 were 3.1% THC and only 0.3% CBD (22). However, cannabis-based medicine extracts and clinical-grade cannabis contain high quantities of CBD, which frequently equal the percentage of THC (23). CBD modifies the effects of THC and is described to have anti-anxiety effects (24) and anti-psychotic benefits (25) and to inhibit the CYP-P450 mediated conversion of THC to 11-OH-THC (26). Furthermore, anticonvulsive and anti-inflammatory effects were reported (27).

More than 30 metabolites of CBD were identified in urine, with the hydroxylation of the 7-methyl group and subsequent oxidation to the corresponding carboxylic acid as the main metabolic route in analogy to THC (14,28,29). The time course of the CBD concentration in plasma after oral application was measured in three studies (8,14,30). It was found that CBD is eliminated in an at least two-phase kinetic with a terminal half life of 31 ± 4 h after smoking (8). The formation of THC from CBD neither occurs by heat during smoking (29) nor by human metabolism.

CBN is not a biosynthetic product of cannabis plants but is formed by air oxidation from THC during preparation of hashish or during longer storage of cannabis products (31). Its average content was 0.3% in marijuana and 1.7% in hashish samples (22). No psychoactive effects of this compound were noted, but it may modify the effects and the metabolization of THC (30). Pharmacokinetic data of CBN are known from smoking, oral application, and infusion (14,32). CBN is not formed from THC in human metabolism. Its main metabolic pathway is the hydroxylation of the C-atom 7 and subsequent transformation to the corresponding carboxylic acid (14,33).

Because of these properties, the determination of CBD and CBN in addition to THC and its metabolites after consumption of cannabis products is interesting from a forensic point of view and in clinical studies. The methods described in literature for this purpose did either not involve all five substances in one run (33–41) or were not sufficiently sensitive (42). In this paper, we describe the development and optimization of a gas chromatography–mass spectrometry (GC–MS) method for the simultaneous and sensitive analysis of THC, 11-OH-THC, THC-COOH, CBD, and CBN from 1 mL plasma. The method was used in a prospective clinical study in which the concentration versus time profiles of the compounds after oral application of 10 mg THC alone and in cannabis extract were measured. Some examples of this application are shown in this paper.

Materials and Methods

Volunteers and plasma samples

In the prospective, double-blind, placebo-controlled cross-over study each of 24 volunteers (age 18 to 45 years) were administered four capsules with either cannabis extract (2.5 mg THC and 1.35 mg CBD per capsule), THC (2.5 mg per capsule), or placebo in fasting state (last meal at least 8 h before application) that lasted for 4 h after drug intake. Between the three applications, there was a one-week wash-out phase. Before each administration, the urine of the volunteers was controlled by EMIT for cannabinoids and other drugs or drug metabolites. Blood samples were taken 30 min before and 30, 60, 120, 180, 240, 360, 540, and 1440 min after drug administration with EDTA as anticoagulant. The samples were centrifuged within 15 min, and the plasma (2–2.5 mL) was separated and frozen at –30°C until investigation.

This study was approved by the local Ethics Committee of the University Hospital Charité of the Humboldt-University Berlin, and written informed consent was obtained from each volunteer.

Reference substances and reagents

Standard solutions (0.1 mg/mL in methanol) of THC, 11-OH-THC, and THC-COOH as well as of the corresponding deuterated compounds (THC-d_3, 11-OH-THC-d_3, and THC-COOH-d_3) were purchased from Promochem (Wesel, Germany). CBD and CBN (1 mg/mL in methanol) as well as the reagents for silylation *N,O*-bistrimethylsilyltrifluoroacetamide (BSTFA, with 1% trimethylchlorosilane), methyltrimethylsilyltrifluoroacetamide (MSTFA, with 1% trimethylchlorosilane), tri-i-propylchlorosilane (TIPS-Cl), methyl-*tert*-butyldimethylsilyltrifluoroacetamide (MTBSTFA), and dimethylphenylchlorsilan were obtained from Sigma-Aldrich (Steinheim, Germany). Trimethylsulfonium hydroxide (TMSH, 0.2M in methanol) and tetramethyloxonium tetrafluoroborate (Me_3OBF_4) were purchased from Macherey & Nagel (Düren, Germany) and from Merck Eurolab (Darmstadt, Germany), respectively. All solvents and other reagents were obtained in analytical grade purity from Merck (Darmstadt, Germany).

Instruments

The GC–MS measurements were performed with a gas chromatograph 6890, a mass selective detector 5973, and an autosampler 7673 (Agilent Technologies GmbH, Waldbronn, Germany).

The solid-phase extraction (SPE) was automatically carried out with a RapidTrace extractor (Zymark, Rüsselsheim, Germany). All steps of column conditioning, sample extraction, washing, and drug elution were monitored by the software RapidTrace Workstation, Version 1.20. For liquid–liquid extraction, a thermomixer 5433 (Eppendorf, Hamburg, Germany) was used. The solvents were removed by an evaporator of the firm Liebisch (Bielefeld, Germany).

SPE and derivatization

Each sample was analyzed twice. After optimization, the following procedure was used: 10 µL of a solution of the internal standards THC-d_3, 11-OH-THC-d_3, and THC-COOH-d_3 (each 1 µg/mL in *n*-propanol) and 1 mL 0.1M acetate buffer pH 4.0 were added to 1 mL plasma in a 10-mL vial and thoroughly mixed. Small amounts of precipitate were removed by centrifugation, and the supernatant was transferred into a 4-mL sample vial of the RapidTrace.

The extraction was carried out with Chromabond C_{18} ec columns (Macherey-Nagel, Düren, Germany, 200 mg extraction

material, 3 mL volume). The columns were conditioned with 6 mL H_2O (0.1 mL/s), 6 mL methanol (0.03 mL/s), and 2 mL acetate buffer pH 4.0 (0.1 mL/s). The extraction of the sample was carried out with a flow rate of 0.02 mL/s. After loading, the columns were washed with 1 mL 0.1M acetic acid in H_2O (0.05 mL/s) and 2 mL 40% acetonitrile/H_2O (0.05 mL/s) and dried for 2 min in a nitrogen stream. Finally, the drugs were eluted with 1.8 mL acetonitrile (0.02 mL/s).

The solvent was evaporated at 40°C in a nitrogen stream, and the residue was derivatized with 20 µL BSTFA for 30 min at room temperature. The mixture was transferred into a 100-µL vial, and 2 µL was injected for GC–MS analysis.

GC–MS measurements

The GC separation was carried out with a capillary column HP 5 MS (95% dimethylsiloxan, 5% diphenylsiloxan, 30 m × 0.25 mm × 0.25 µm) with the following temperature program: 2 min at 130°C, 20°/min up to 300°C, and 5 min at 300°C. The temperatures of the injector, the interface, the ion source, and the quadrupole were 290°C, 310°C, 260°C, and 106°C, respectively. The injection mode was spitless, and the injection volume was 2 µL.

The retention times under these conditions and the *m/z* values chosen for the detection of the trimethylsilyl derivatives in the selected ion monitoring mode (SIM, five time windows) are given in Table I. A typical chromatogram of a plasma sample spiked with each 5 ng/mL of the five analytes and 10 ng/mL of the deuterated standards is shown in Figure 1. For confirmation of the peak identity, the exact retention times and the peak-area ratios of the three *m/z* traces per substance were controlled, and those for THC, 11-OH-THC, and THC-COOH compared with those of the deuterated standards.

Calibration and validation of the method

The extraction recoveries of the sample preparation were calculated as the quotient of the GC–MS peak areas obtained from spiked plasma samples after extraction and by direct injection of the same substance amount. In this case, the internal standards were added after the extraction in order to correct for fluctuations of the GC–MS sensitivity. The mean recoveries obtained from two extraction experiments are given in Table II.

The calibration and the validation of the method were performed according to the recommendations of the program Valistat (43). Plasma samples were spiked with 0.0, 0.25, 0.5, 1.0, 1.5, 2.0, 5.0, and 10.0 ng/mL of the five analytes. For THC-COOH, 25 ng/mL and 50 ng/mL were additionally included because of the expected higher concentrations. These samples were extracted, derivatized, and measured by GC–MS as described previously. Each concentration was prepared and analyzed six times. The peak areas of the quantifier ion traces of THC, 11-OH-THC, and THC-COOH (Table I) were related to those of the corresponding deuterated internal standards. 11-OH-THC-d_3 (*m/z* 374) was also used as internal standard for CBD and CBN. The calibration curves were linear with zero intersections for all five compounds. The correlation coefficients and the limits of detection (LOD) and quantification (LOQ) determined from these data according to the validation program Valistat (43) are given in Table II. In this statistical program, the standard deviation of the calibration curve is used for calculation of LOD and LOQ. About the same values were estimated from the 3-fold and the 10-fold standard deviation of the baseline noise. The LOD and LOQ estimated on this basis from real samples of the pharmacokinetic study were, in many cases, below those given in Table II.

For determination of the interday reproducibility of the method, spiked serum samples containing 10 ng/mL of the five substances were analyzed in each of the 40 measurement series of the study. The apparent recoveries and standard deviations calculated from these measurements are also given in Table II. Furthermore, all plasma samples of the clinical study were measured twice. The average deviation of the concentrations from the mean of both concentrations was between 1.8 and 3.9% (Table II).

Table I. Retention Times, Time Windows, and Mass-to-Charge Ratio Values for the Detection of Trimethylsilyl Derivatives of Cannabinoids in the SIM Mode

Compound	*m/z* Registered in SIM Mode*	Retention Time (min)†	Time Window (min)
CBD-2TMS	**319**, 390, 458	9.45	9.10–9.80
THC-TMS-d_3	306, 374, **389**	9.97	9.80–10.15
THC-TMS	303, 371, **386**	9.98	9.80–10.15
CBN-TMS	**310**, 367, 382	10.36	10.15–10.60
11-OH-THC-2TMS-d_3	**374**, 462, 477	10.98	10.60–11.20
11-OH-THC-2TMS	**371**, 459, 474	10.99	10.60–11.20
THC-COOH-2TMS-d_3	**374**, 476, 491	11.65	11.20–12.00
THC-COOH-2TMS	**371**, 473, 488	11.66	11.20–12.00

* Quantification ions are bold.
† Depends on column length.

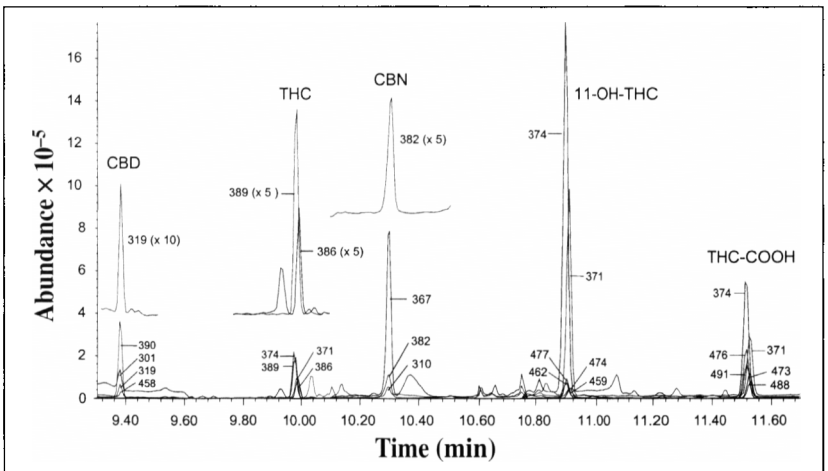

Figure 1. GC–MS-SIM chromatogram of a plasma sample spiked with each 5 ng/mL of THC, 11-OH-THC, THC-COOH, CBD, and CBN and 10 ng/mL of the internal standards THC-d_3, 11-OH-THC-d_3, and THC-COOH-d_3.

Results and Discussion

The analysis of plasma samples for cannabinoids by GC–MS is daily routine in forensic toxicological laboratories. Normally, the investigations are limited to THC, 11-OH-THC, and THC-COOH and detection limits of 1 ng/mL are sufficient. However, in the present study, CBD and CBN had to be included, and a detection limit below 0.5 ng/mL was necessary in order to follow up the concentrations for a sufficiently long time after administration. Only 2–2.5 mL plasma per sample were available. Therefore, the method, particularly the extraction and the derivatization, had to be adapted to these requirements before application to the study.

Table II. Data of the Calibration, Recoveries of Extraction, LOD and LOQ, Apparent Recoveries, and Reproducibility of the Method for Determination of Cannabinoids*

	THC	11-THC-OH	THC-COOH	CBD	CBN
F	0.958	1.084	1.047	0.00564	0.0045
R^2	0.9996	0.9997	0.9988	0.9977	0.9968
Recovery of extraction (%)	50	95	85	90	43
LOD (ng/mL)	0.24	0.15	0.26	0.29	1.1
LOQ (ng/mL)	0.80	0.51	0.88	0.95	3.9
Apparent recovery (n = 40) (%)†	101	99	95	104	101
Standard deviation (n = 40) (%)	2.8	1.9	3.7	2.9	7.7
Average deviation from mean in double determination (n = 480) (%)	2.9	1.8	3.0	3.9	–‡

* Abbreviations: F = calibration factor (c = F × peak-area ratio analyt/internal standard), R = correlation coefficient, LOD = limit of detection, LOQ = limit of quantification.
† The terms "recovery" and "apparent recovery" were used according to the IUPAC recommendation published in (48). The apparent recoveries (%) are amount calculated from the calibration curve/spiked amount × 100.
‡ CBN was not detected in the samples of the clinical study.

Development of the analytical method

Derivatization. At first, a suitable derivatization reaction had to be found. This was also a prerequisite for the extraction optimization. For this purpose, different derivatization possibilities were compared. Mixtures of 100 ng of each of the five compounds were prepared from the standard solutions by evaporation of the solvent, and the reagents were applied to the residues. The products were analyzed by GC–MS in SCAN mode. In those cases in which all five compounds were clearly detected and well separated, the experiments were repeated with 5, 2, 1, and 0.5 ng of the substances, and the measurements were performed in SIM mode. The results of these experiments are shown in Table III.

Table III. Comparison of Different Derivatization Methods for Cannabinoids*

Reagent/Method*	CBD	THC	CBN	11-OH-THC	THC-COOH
No derivatization	+	+	+	–	–
Methylation (49)					
MeI/TBAH in DMSO	–	+	+	+	+
MeI/TBAH in DMSO + TMSH	–	+	+	–	–
MeI/NaOH in ACN	+	+	+	+	+
in DMF	+	+	+	–	–
in DMSO	–	+	+	–	–
MeI/K_2CO_3 in DMF	+	+	+	–	–
MeI/$LiAlH_4$ in DMF	–	–	–	–	–
TMSH	+	+	+	+	+
$[Me_3O][BF_4]$ in DCM	–	–	–	–	–
Alkylation + acylation (50)					
PFPA/PFPOH	+	+	+	+	+
Silylation (33,45)					
BSTFA	+	+	+	+	+
MTBSTFA	+	+	+	+	+
TIPS-NEt_2	+	+	+	+	–
Me_2PhSi-NEt_2	+	+	+	–	–
Me_2iPrSi-NEt_2	+	+	+	+	+

* GC-MS-detection in SCAN after treatment of 100 ng standard: –, not detected; +, detected; ACN, acetonitrile; BSTFA, *N,O*-bistrimethylsilyltrifluoroacetamide; DMF, dimethylformamide; DMSO, dimethylsulfoxide; MeI, methyl iodide; Me_2PhSi-NEt_2, *N*-(dimethylphenylsilyl)-*N,N*-diethylamine; Me_2iPrSi-NEt_2, *N*-(dimethyl-i-propylsilyl)-*N,N*-diethylamine; MTBSTFA, methyl-*tert*-butyldimethylsilyltrifluoroacetamide; PFPA, pentafluoropropionic anhydride; PFPOH, pentafluoropropanol; TBAH, tetrabutylammonium hydroxide, TIPS-NEt_2, tri-i-propylsilyl-diethylamine; and TMSH, trimethylsulfonium hydroxide.

Without derivatization, 11-OH-THC and THC-COOH could not be detected, and the sensitivity and chromatographic properties of the other three compounds were not sufficient.

The methylation is a preferred derivatization reaction for cannabinoids (37,39,41). The reaction with methyl iodide (MeI) in dimethyl sulfoxide (DMSO) and tetrabutylammonium hydroxide (TBAH) as phase transfer catalyst and subsequent extraction with isooctane was not successful for CBD. Furthermore, the detection limits of about 1 ng/mL for the other four compounds were not sufficient. Use of acetonitrile (ACN) or dimethylformamide (DMF) instead of DMSO and NaOH, K_2CO_3, or $LiAlH_4$ instead of TBAH did not improve the results. Only in the reaction with MeI in NaOH/acetonitrile were all five compounds derivatized, but again with too small sensitivity.

The experiments with trimethyloxonium tetrafuoroborate (Meerwein salt) as methylation agent in dichloromethane (44) were unsuccessful too. For the application of this reagent, extremely dry conditions are required, which obviously were not fulfilled in usual routine analysis. However, all five compounds were methylated by TMSH (0.2M in methanol) (41). With this reagent, the methylation occurs at high temperature in the injector. Because this method is very convenient for routine measurements, some experiments for optimization were performed. As a result, the detection limits of 3 to 4 ng/mL were not sufficient, and for THC-COOH, four peaks possibly caused by incomplete or slow reaction of the OH and the COOH group of this compound appeared in the chromatogram. Experiments to apply this reagent to the products of the MeI/TBAH in DMSO reaction in a two-step derivatization decreased the detectability of 11-OH-THC and THC-COOH and did not lead to the detection of CBD.

By combined alkylation and acylation with a mixture of pentafluoropropionic anhydride and pentafluoropropanol (PFPA/PFPOH) in analogy to the procedures described in literature (38,40), all five substances were detected. However, THC and CBD gave the same peak of a monoacylated product and could not be distinguished in the chromatograms. Furthermore, in contrast to literature data with GC–negative ion chemical ionization-MS (38), the sensitivity was not sufficient in our experiments using GC–EI-MS.

Silylation is most frequently used for derivatization of cannabinoids (33,35,36,42,45) because OH and COOH groups react equally well. Five different silylation reagents were tested: BSTFA, MTBSTFA, tri-i-propylsilanyl-diethylamine, phenyl-dimethylsilyl-diethylamine, and i-propyl-dimethylsilyl-diethylamine (i-$PrMe_2Si$-NEt_2). The last three reagents were prepared from the corresponding chlorosilanes and diethylamine (45). Equally good results were obtained with BSTFA, MTBSTFA, and i-$PrMe_2Si$-NEt_2 for all five cannabinoids. Because of its higher stability and commercial availability, BSTFA was chosen for the further experiments. This reaction was tested at different temperatures, reaction times, and with different amounts of reagent. Best results were obtained with 20 µL BSTFA after 30 min at room temperature. The EI-mass spectra of the trimethylsilyl derivatives were in agreement with literature data (33), and the mass-to-charge ratios selected for the SIM detection are shown in Table I.

Extraction. In the literature, liquid–liquid extraction (33,40–42) as well as SPE procedures (35–39) for analysis of cannabinoids from blood are described. Before extraction, 1 mL of the plasma sample was diluted with acetate buffer (pH 4.0). This pH proved to be optimal because the extraction recoveries of THC-COOH and of CBN from more basic solution (e.g., pH 6.0) decreased and the analytes are less stable in a more acidic solution.

Liquid–liquid extraction experiments were carried out with methyl-*t*-butylether, ethyl acetate, 1-chlorobutane, and isooctane. Methyl-*t*-butylether and ethyl acetate were not suitable because too many matrix components were extracted. On the other hand, with the non-polar isooctane, THC-COOH was not sufficiently extracted. With 2 mL 1-chlorobutane, all five compounds were extracted and the chromatograms were less disturbed by matrix components, but only medium or low extraction recoveries were found: 43% THC, 59% 11-OH-THC, 11% THC-COOH, 65% CBD, and 46% CBN.

Therefore, an automatic SPE procedure with Chromabond C_{18} ec columns (200 mg C_{18}-modified silica) at the extractor RapidTrace was chosen (see Materials and Methods section). The method recommended by Varian (46) was optimized with respect to the amount and composition of the wash solution (2 mL 40% acetonitrile/H_2O) and the solvent for elution (1.8 mL acetonitrile). With this method, the extraction recoveries were between 43% and 95% (Table II), and the disturbance by matrix components was sufficiently low.

Validation of the method. The total procedure was validated according to the criteria of the Excel®-based program "Valistat" (43). The details are described in the Materials and Methods section, and the data are shown in Table II. With the exception of CBN, the LODs were between 0.15 and 0.29 ng/mL. The higher LOD of CBN (1.1 ng/mL) is caused by the lower extraction yield and by the use of the less intense CBN-TMS fragment *m/z* 310 for quantification. Particularly at low concentrations, the most intense fragment (*m/z* 367) was disturbed by the matrix, and the calibration with *m/z* 382 was not sufficiently linear.

A high intraday reproducibility was proven by the mean deviation of 1.8–3.9% (n = 384) between the two measurements of all samples of the clinical study. The interday reproducibility of the method was calculated from a control solution measured in each analysis series (standard deviation of 2.9–5.5%, n = 40). The apparent recovery of the five compounds (concentrations 10 ng/mL) was between 95 and 104%.

Altogether, the method proved to be sufficiently sensitive, accurate, and reproducible for the investigation of plasma concentrations after low oral cannabis doses.

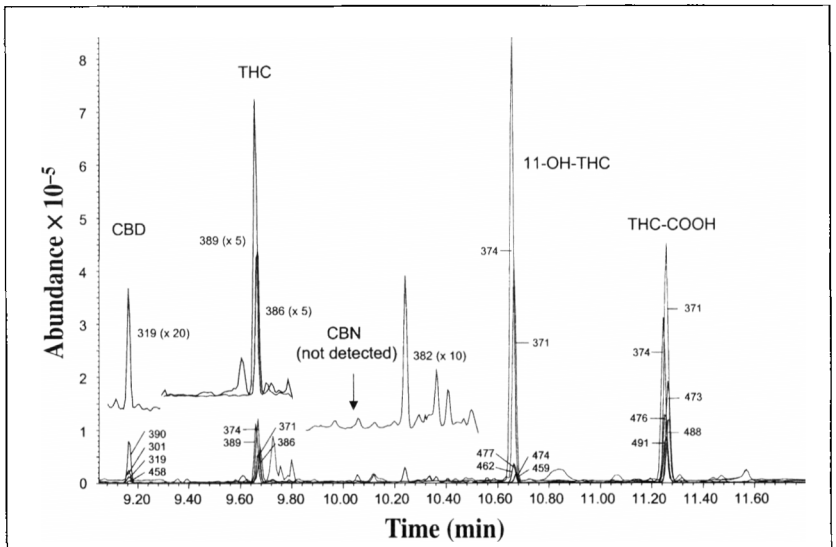

Figure 2. GC–MS-SIM chromatogram of a plasma sample of a volunteer collected 60 min after application of a capsule with cannabis extract containing 10 mg THC and 5.4 mg CBD. The concentrations in this sample were THC, 5.5 ng/mL; 11-OH-THC, 4.4 ng/mL; THC-COOH, 13 ng/mL; and CBD, 2.3 ng/mL. CBN was not detected.

Plasma concentrations after a single oral application of 10 mg THC and of cannabis extract containing 10 mg THC and 5.4 mg CBD

In the double blind clinical study, plasma samples were obtained from each volunteer before and at eight different times after the application of a capsule with either placebo, 10 mg THC or cannabis extract (10 mg THC + 5.4 mg CBD). Altogether 648 plasma samples were investigated, and each sample was analyzed twice. A typical chromatogram of a plasma sample collected 60 min after application of a cannabis extract capsule is shown in Figure 2. THC, 11-OH-THC, THC-COOH, and CBD are clearly detected. CBN was not found in the samples. This was not only caused by the higher LOD of this compound because

it was shown by high-performance liquid chromatography that no essential amounts of this compound were present in the cannabis extract of the capsules.

Table IV. Maximum Concentrations (C_{max}) of THC, 11-OH-THC, THC-COOH, and CBD and Corresponding Time (t_{max}) After Administration*

	THC (n = 24)	11-OH-THC (n = 24)	THC-COOH (n = 24)	CBD (n = 24)
Administration of 10 mg THC				
C_{max} (ng/mL) (range)	0.67–7.99	1.12–11.14	12.03–57.63	–
C_{max} (ng/mL) (mean)	3.20	4.48	32.9	–
t_{max} (min) (range)	30–183	30–190	67–235	–
t_{max} (min) (mean)	63.6	90	124	–
Detection time after intake (min)	240–360	360–540	> 1440	–
Administration of cannabis extract containing 10 mg THC + 5.4 mg CBD				
C_{max} (ng/mL) (range)	1.18–10.27	1.83–12.34	19.2–70.6	0.30–2.57
C_{max} (ng/mL) (mean)	4.05	4.88	35.46	0.95
t_{max} (min) (range)	33–125	37–130	65–230	30–120
t_{max} (min) (mean)	56	81.9	115	59.6
Detection time after intake (min)	240–360	360–540	> 1440	180–240

* C_{max}: highest measured concentrations. t_{max}: estimated from the curves obtained by spline fitting to the measured points.

The maximum concentrations and the times (t_{max}) at which these concentrations were attained are given in Table IV. The t_{max} values were estimated from the curves obtained by spline fitting to the measured points. Furthermore, the time periods for the detection of the compounds after intake are shown.

The concentration versus time curves of THC and its metabolites 11-OH-THC and THC-COOH are characteristic for oral intake of the drug (4,14,16–19). Four typical examples are shown in Figure 3. In contrast to smoking, the concentrations of THC-COOH were from the beginning higher than that of THC. As a rule, after 1 h the concentrations of 11-OH-THC were also higher than those of THC, and this metabolite could be detected for a longer time than THC.

But there is a large interindividual variation of the concentrations and of the concentration versus time curves. In most cases, the THC peak concentration was attained between 30 and 60 min after drug intake with a steady decrease after this time (Figure 3A). The maximum concentration of 11-OH-THC was about 15–30 min later and that of THC-COOH about 30–70 min later attained than that of THC. However, in a few cases, a delayed resorption was found with a non-detectable or very small THC concentration in the 30 and 60 min samples and reaching the maximum 120 or 180 min after drug intake (Figure 3C). In some other cases an intermittent resorption with a shoulder or a second maximum was observed (Figures 3B

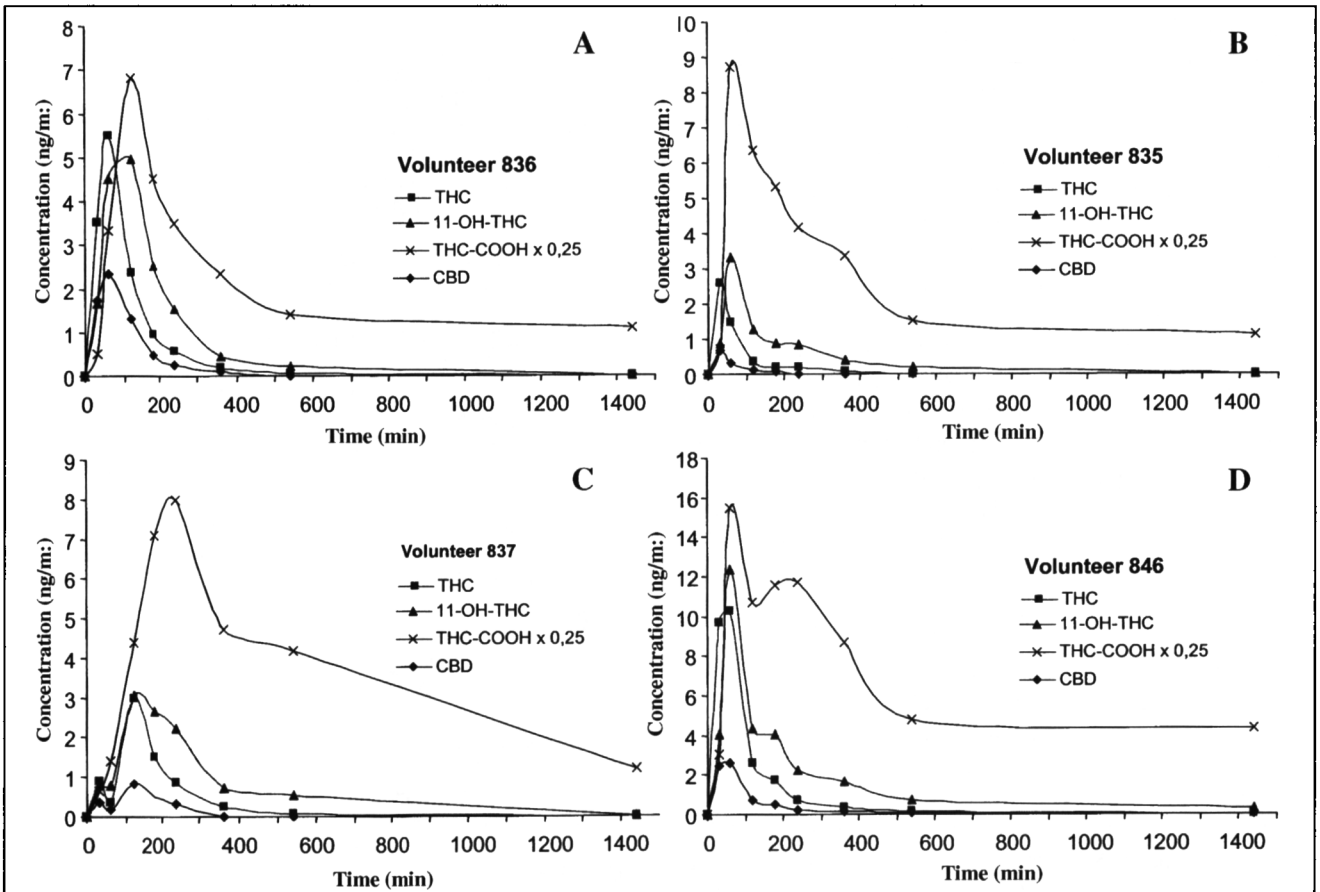

Figure 3. Plasma concentration versus time curves obtained after administration of a single capsule of cannabis extract containing 10 mg THC and 5.4 mg CBD: optimal resorption (A), intermittent resorption with a second maximum for THC and 11-OH-THC (B); delayed onset resorption (C); and shoulder for THC and 11-OH-THC and second maximum for THC-COOH (D).

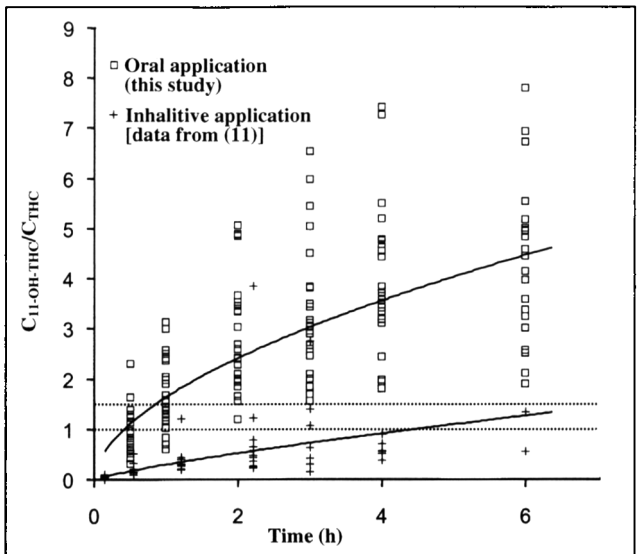

Figure 4. Comparison of the concentration ratio 11-OH-THC/THC between oral intake (this study, 10 mg THC) and smoking of a 1.75% or a 3.55% marijuana cigarette [data from Huestis et al. (10)].

and 3D). The same irregularities were frequently seen in the curves of both metabolites and of CBD of the same volunteer. A similar variability of the resorption kinetics was already observed in a previous study of the authors (47).

The rapid increase of the metabolite concentrations is caused by the extensive first-pass metabolism of THC, which is specific for oral intake. This is particularly the case for the primary metabolite 11-OH-THC. Therefore, the concentration ratio 11-OH-THC/THC could be a criterion for distinguishing between oral and inhalative drug consumption. This ratio is compared in Figure 4 with the results obtained by Huestis et al. (10) in smoking experiments with 6 volunteers and joints containing 1.75% and 3.5% THC over a period of 6 h after consumption. As expected, the ratio 11-OH-THC/THC increases for both kinds of drug use with time. However, the mean values of the oral administration are at all times by a factor of 3 to 4 higher than those of smoking. Despite the large variation of the ratios within both groups, there is almost no overlapping of the data apart from some outliers. The same results were obtained for the cannabis extract administration. It can be concluded from the results given in Figure 4 that within the first 2 h after consumption, a ratio 11-OH-THC/THC > 1 and later than 2 h after consumption, a ratio 11-OH-THC/THC > 1.5 are strong indications for oral intake of THC. The content of CBD in cannabis products varies in a wide range (21–23). The ratio of CBD/THC of 0.54 administrated in the present study is relatively high in comparison to the confiscated samples described by ElSohly et al. [mean 0.096 (22)]. Nevertheless, the dose of 5.4 mg CBD is in a range that can be assumed in cannabis abuse and particularly also in cannabis-based medicine extracts (23). Data for CBD concentrations in blood after controlled administration of this compound are rare. Ohlsson et al. (8) measured 37–61 ng/mL (n = 3, mean 48.4 ng/mL) 1 h after intravenous injection of 20 mg and 3.0–17.8 ng/mL (n = 3, mean 10.2 ng/mL) 1 h after smoking of 19.2 mg deuterium-labeled CBD. Agurell et al. (30) found 1.1–11 ng/mL 1 h after oral application of 40 mg CBD (n = 12, mean 5.5 ng/mL) in chocolate cookies. The concentrations determined in the present study (0.30–2.57 ng/mL, mean 0.95 ng/mL 1 h after oral intake of 5.4 mg, see Table IV) are in agreement with these data.

In this study, CBD was detectable between 3 and 4 h after administration. Generally, the detection of CBD should be favourable as an additional proof of cannabis exposure and for an improved interpretation of THC effects with consideration of the modifying interaction by CBD (23–25).

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