International Journal of Legal Medicine
https://doi.org/10.1007/s00414-018-01994-y

ORIGINAL ARTICLE

Cannabinoid concentrations in confiscated cannabis samples and in whole blood and urine after smoking CBD-rich cannabis as a "tobacco substitute"

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Received: 2 October 2018 / Accepted: 19 December 2018

Abstract

In Switzerland, only cannabis with a total Δ^9-tetrahydrocannabinol (THC) content higher than 1% is controlled by the narcotics legislation. Cannabis products rich in cannabidiol (CBD) and low in THC can be legally sold as tobacco substitutes. In this paper, we address analytical and forensic toxicological issues related to the increasing availability and consumption of these products. Based on the analysis of 531 confiscated cannabis samples, we could establish classification thresholds: plant material with a ratio of total THC/total CBD ≥ 3 is graded as THC-rich/CBD-poor, whereas samples with a ratio ≤ 0.33 are categorized as CBD-rich/THC-poor cannabis. We also evaluated an on-site test kit as a rapid alternative to the laborious liquid or gas chromatography (LC or GC)-based techniques normally used for the differentiation between THC- and CBD-cannabis. Furthermore, we determined whole blood and urine cannabinoid levels after smoking different doses of legal CBD-cannabis. A male volunteer smoked one cigarette within 15 min and four cigarettes within 1 h and within 30 min, respectively. Cigarettes contained on average 42.7 mg CBD and 2.2 mg THC. Blood samples were collected up to 1.1 h and urine samples up to 27.3 h after the beginning of smoking. All urine samples tested negative by three immunochemical assays for detection of cannabis use. This is an important finding for abstinence monitoring. However, we found that the trace amounts of THC present in CBD-cannabis can produce THC blood levels above the Swiss legal limit for driving, and thus render the consumer unable to drive from a legal point of view.

Keywords Tetrahydrocannabinol · Cannabidiol · Classification · Drug of abuse testing · Driving while impaired

Introduction

For centuries, cannabis has been cultivated as a source of fiber, food, and oil, as a medicinal plant as well as for recreational purposes [1]. To date, well over 400 constituents have been identified in cannabis, of which at least 70 are phytocannabinoids, a group of 21 terpenophenolic compounds to which the majority of the plant's biological activities have been ascribed [2]. Δ^9-tetrahydrocannabinol (THC) is generally accepted to be the compound responsible for the psychoactive properties of cannabis inducing euphoria, altered sensory perception and relaxation, also known as the "high" that recreational cannabis users seek. Additionally, THC has been found to have a number of therapeutic attributes including anti-inflammatory, antiemetic, appetite stimulant, analgesic, and antispasmodic effects [3]. However, THC has also been associated with a number of adverse effects, including tachycardia, anxiety, cognitive deficits, paranoia, as well as with an increased risk of developing chronic psychosis and dependence [4–7]. Since several years, other cannabinoids have come into the focus of research and legislation, the most prominent being cannabidiol (CBD), which is non-euphoriant and is thought to modulate the psychoactivity of THC, thus alleviating the adverse effects of THC. Furthermore, CBD has shown promising therapeutic activity per se, such as anti-inflammatory, anticonvulsive, anxiolytic, analgesic, neuroprotective, anticancer, and antioxidant effects [8, 9].

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Published online: 05 January 2019

The cannabinoid composition of the cannabis plant material, i.e., the ratio of the different cannabinoids, is primarily determined by genetic factors (strain type), but is also affected by environmental and harvesting conditions (e.g., growing and storage conditions, state of maturity at harvest) [10]. Over the last few decades, recreational users seeking the ultimate "high" have selectively bred cannabis plants to produce highest THC and lowest CBD levels and until recent years, little effort has been made to breed plants with high CBD levels as there was no demand among recreational cannabis users. However, due to the growing interest in the therapeutic effects of CBD, cannabis breeders have been working to create new strains rich in CBD. Varieties expressing up to 25% total CBD and less than 1% total THC (typically 0.3–0.7%) within the floral tissue have recently been bred [11]. The total CBD and total THC content (in % of dry weight) corresponds to the sum of the free cannabinoid and its pharmacologically inactive carboxylic acid precursor (CBD-acid and THC-acid A, respectively), corrected for weight of the carboxylic acid groups. Decarboxylation of the acids into their active neutral forms occurs spontaneously as the plant ages and, to a much larger extent, upon heating (e.g., upon smoking, vaporizing, or baking the plant material) [12].

In the European Union, cannabis plants containing no more than 0.2% total THC and producing no psychoactive effects can be legally cultivated for fiber and seeds production. In Switzerland, only plant material with a total THC content of 1% or higher is controlled by the narcotics legislation [13]. CBD-rich/THC-poor cannabis products have been on the Swiss legal market as tobacco substitutes for more than a year and their consumption has recently become increasingly popular, for both therapeutic and recreational purposes.

Since 2017, our laboratory has analyzed over 800 cannabis samples confiscated by Swiss police forces by a fully validated high-performance liquid chromatography-diode array detector (HPLC-DAD) method for determination of the cannabinoid profile [14]. In this work, we will give a representative overview on the concentrations of CBD and THC in the confiscated material and we will propose a ratio of total THC/total CBD to discriminate between CBD- and THC-cannabis on an analytical level. Furthermore, we will evaluate the performance of a recently introduced on-site test kit [15] for the rapid distinction between CBD- and THC-cannabis.

As strategy to prevent cannabis-impaired driving, Switzerland, among several other European countries [16], has adopted a zero tolerance law that prohibits driving with any detectable amount of psychoactive THC in the blood. The detection of THC in blood is deemed proof of the driver's acute impairment and inability to drive. According to the directives of the Federal Roads Office an analytical cut-off of 1.5 μg/L is employed for the reliable detection of THC in whole blood. In forensic practice, a confidence interval of 30% is taken into consideration, resulting in an effective cut-off of 2.2 μg/L (2.2 μg/L minus 30% is 1.54 μg/L, which is just higher than the analytical cut-off of 1.5 μg/L). Since CBD-rich cannabis products contain trace amounts of THC, the question arises whether their consumption might cause THC blood levels ≥2.2 μg/L, and thus render the consumer unable to drive from a legal point of view. Another analytical question commonly raised is whether smoking legal CBD-rich cannabis can produce urinary cannabinoid levels high enough to result in positive findings in immunological urine drug tests. Urine drug screening by immunochemical assays is often performed in cases of suspected drug-impaired driving, for workplace drug testing, and to monitor abstinence from drug use [17, 18]. Antibodies used in urinary immunochemical tests for detection of cannabis use primarily target the THC metabolite 11-nor-9-carboxy-THC (THCCOOH) and have variable degrees of cross-reactivity with other metabolites and cannabinoids [19].

Herein, we attempt to answer the aforementioned questions by reporting whole blood and urine cannabinoid levels measured in a male subject after smoking different doses of CBD-rich/THC-poor cannabis flowers.

Material and methods

Chemicals and reagents

Dried CBD-rich/THC-poor cannabis flowers (*Cannabis sativa* L.) were obtained from Swiss Cannabis SA (Härkingen, Switzerland). Acetonitrile (HPLC gradient grade, 99.9%) was purchased from Acros Organics (Geel, Belgium) and methanol (absolute, HPLC grade) from Biosolve (Dieuze, France). Butyl acetate (HPLC grade, 99.7%), formic acid solution (puriss p.a., 50% in water), and *E. coli*-type IX-A β-glucuronidase were obtained from Sigma-Aldrich (Buchs, Switzerland). Potassium dihydrogen phosphate (puriss p.a., ACS), di-sodium hydrogen phosphate dihydrate (analytical grade), and *H. pomatia* β-glucuronidase/arylsulfatase were acquired from Merck (Darmstadt, Germany). Deionized water was prepared in-house with a Direct-Q water purification system from Millipore (Zug, Switzerland). THC, 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), and CBD reference standards as well as their trideuterated analogs were purchased from Cerilliant (Round Rock, TX, USA). THC-acid A, CBD-acid, and cannabinol (CBN) reference standards were obtained from Lipomed (Arlesheim, Switzerland). Blank human blood and urine samples were obtained from the blood donor center in Bern, Switzerland and donated by a volunteer, respectively, and were tested for the absence of cannabinoids prior to use.

Colorimetric on-site test kit for differentiation of CBD- versus THC-cannabis

For differentiation of CBD- versus THC-cannabis, a liquid colorimetric test can be used by police forces on the street and on indoor or outdoor cultivation sites, or as preliminary tests in the laboratories. A commercial test kit is already in use by several Swiss police forces [15]. The kit contains two solutions: solution 1, consisting of 0.2 mg/mL 4-aminophenol in ethanol/isopropanol (95:5; *v*/*v*), which was acidified with 0.5% hydrochloric acid (HCl 2 N); solution 2, consisting of 30 mg/mL sodium hydroxide in ethanol/water (70:30; *v*/*v*).

To conduct the differentiation, a small sample of dried or fresh cannabis plant material is transferred into a glass vial and 1–2 mL of solution 1 (depending on sample size) and four drops of solution 2 are added. The vial is closed, shaken vigorously and left to stand for 2 min to allow reaction. Subsequently, the color of the solution is visually evaluated: a blue coloration indicates THC-rich cannabis; a pink coloration indicates CBD-rich cannabis. For quantitative determination of cannabinoids, the HPLC-DAD method, which is described hereafter, is used.

HPLC-analysis of cannabis products for determination of THC, THC-acid A, CBD, CBD-acid, and CBN

The HPLC-DAD method for the analysis of cannabis products has already been published [14] and has recently been updated by adding CBD-acid to the calibrators and the control samples, in the same concentrations as THC-acid A.

Data treatment and evaluation of confiscated cannabis samples

Since January 2017, 808 confiscated samples have been analyzed for cannabinoids at our institute. For the evaluation of the CBD and THC content, only samples containing dried or fresh cannabis flowers were considered. Samples consisting of (burnt) joint material, plant material without flowers, hashish, or oil were excluded. Furthermore, 119 samples were analyzed before CBD-acid had been included in the method and were therefore also excluded as no re-analysis was possible. Finally, 531 confiscated samples have been evaluated with respect to CBD and THC concentrations. Data evaluation and visualization were conducted using R version 3.5.1 [20].

Study design for the smoking experiment

A healthy male subject (42 years, 75 kg, 183 cm; regular tobacco cigarette smoker) participated in three experimental sessions, separated by 3 weeks, in which he smoked either one cigarette ad libitum for up to 15 min (session A); four cigarettes within 1 h (session B); or four cigarettes within 30 min (session C) containing 0.5 g of a 1:1 mixture of CBD-rich cannabis flowers and tobacco. The smoked amounts of CBD and THC averaged about 42.7 and 2.2 mg, respectively per cigarette. Whole blood samples were collected between cigarettes and up to 1.1 h and urine samples up to 27.3 h after the beginning of smoking.

Qualitative analysis of urine samples

Qualitative screening by homogeneous enzyme immunoassay was performed with 300 μL of urine on an AU480 analyzer (Beckman Coulter, Nyon, Switzerland) using the Immunalysis HEIA Cannabinoids kit (Pomona, CA, USA) according to the manufacturer's instructions. Furthermore, all urine samples were qualitatively analyzed for cannabinoids using the THC lateral flow immunoassay test and automatic P.I.A.^{2} readout system from Protzek (Lörrach, Germany) as well as with the Drug-Screen-Multi 12Z dip test from Nal von Minden (Moers, Germany). All three immunochemical assays were calibrated with the THC metabolite THCCOOH (50 μg/L cut-off for THCCOOH).

Quantitative analysis of blood and urine samples

Sample preparation

Two hundred microliters of whole blood and urine samples were spiked with 20 μL of internal standard solution (2 ng THC-d_3 and 11-OH-THC-d_3, 10 ng THCCOOH-d_3, 2 ng CBD-d_3) and processed by protein precipitation with 600 μL of acetonitrile for determination of free cannabinoid concentrations or by β-glucuronidase treatment followed by liquid-liquid extraction with 1 mL of butyl acetate for determination of total cannabinoid concentrations (free and glucuronidated). For hydrolysis of glucuronide conjugates of THC, 11-OH-THC, and THCCOOH, 10 μL of *H. pomatia* β-glucuronidase/arylsulfatase and 200 μL of 66 mM phosphate buffer pH 6 were added, and the samples were incubated at 47 °C for 2 h. Hydrolysis of CBD-glucuronide was achieved by adding 200 μL of 75 mM phosphate buffer pH 6.8 containing *E. coli*-type IX-A β-glucuronidase at a concentration of 5000 U/mL and incubating at 37 °C for 2.5 h. After mixing and centrifugation, the organic phases (acetonitrile/butyl acetate) were evaporated to dryness at 50 °C under nitrogen and reconstituted in 200 μL of acetonitrile/water/formic acid, 60:40:0.1; *v*/*v*/*v*. Five microliters of the prepared samples were injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

Calibration samples were prepared by addition of reference standard solutions to blank blood and blank urine, respectively, and were processed by protein precipitation as described above. Linear ranges in blood and urine were 0.5–20 μg/L for THC and 11-OH-THC, 2.5–100 μg/L for THCCOOH, and 1–

250 μg/L for CBD. Lower limits of quantification (LLOQ) were 1 μg/L for THC and 11-OH-THC, 5 μg/L for THCCOOH, and 1 μg/L for CBD. Limits of detection (LOD) were 0.5 μg/L for THC and 11-OH-THC, 2 μg/L for THCCOOH, and 0.1 μg/L for CBD.

LC-MS/MS instrumentation

Cannabinoid concentrations were determined using our published column-switching LC-MS/MS method [21] with some modifications. The LC-MS/MS consisted of an UltiMate 3000 HPLC system (Dionex, Olten, Switzerland) coupled to a 4500 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer with a Turbo V ion source (SCIEX, Brugg, Switzerland). Analyst software version 1.6.2 (SCIEX, Brugg, Switzerland) was used for data acquisition and analysis.

LC was performed with a Mercury Synergi 4 μm Polar RP trapping column (20 × 2.0 mm) and a Kinetex 2.6 μm C8 analytical column (50 × 2.1 mm), both obtained from Phenomenex (Torrance, CA, USA). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. A 5-μL aliquot of the prepared sample was loaded onto the trapping column with a 500-μL/min flow of 50% B and by dilution via a T-union with a 300-μL/min flow of mobile phase A. After 1 min, the trapped analytes were eluted in backflush mode to the analytical column by running a gradient from 30 to 97.5% B over 6.5 min at a flow rate of 300 μL/min. After an isocratic hold for 2.5 min, the columns were re-equilibrated for 2 min, resulting in a total runtime of 12 min.

Mass spectrometric data were acquired in positive electrospray ionization and selected reaction monitoring mode, with an ion spray voltage of 3500 V and an ion source temperature of 650 °C. Measured transitions were THC/CBD, m/z 315.2 → 139.1*, 315.2 → 123.0; THC-d_3/CBD-d_3, m/z 318.2 → 196.3*, 318.2 → 123.0; 11-OH-THC, m/z 331.2 → 313.2*, 331.2 → 193.2; 11-OH-THC-d_3, m/z 334.2 → 316.2, 334.2 → 196.2; THCCOOH m/z 345.2 → 327.3*, 345.2 → 299.2; THCCOOH-d_3, m/z 348.2 → 330.2, 348.2 → 302.2. Transitions marked with an asterisk were used for quantification.

Normalization of cannabinoid concentrations in urine

In order to correct for dilution of the urine and thus to facilitate result interpretation, the analyte concentrations were normalized to the creatinine content of the urine by means of the following formula [22]:

$$\mathrm{Conc.}_{\mathrm{CR\ normalized}} = \mathrm{Conc.}_{\mathrm{Sample}} * \frac{\mathrm{CR}_{\mathrm{Ref.}}}{\mathrm{CR}_{\mathrm{Sample}}}$$

$\mathrm{Conc.}_{\mathrm{CR\ normalized}}$ being the creatinine-normalized concentration (μg/L); $\mathrm{Conc.}_{\mathrm{Sample}}$ the analyte concentration (μg/L); $\mathrm{CR}_{\mathrm{Ref.}}$ the creatinine reference concentration, defined as 100 mg/dL; and $\mathrm{CR}_{\mathrm{Sample}}$ the creatinine concentration in the sample (mg/dL), measured spectrophotometrically by Jaffé's reaction on the AU480 analyzer during qualitative screening of the urine samples. Details of the creatinine determination are reported elsewhere [23].

Results

Colorimetric on-site test kit

Several confiscated samples containing dried or fresh cannabis flowers have been tested with the colorimetric on-site kit. The typical coloration difference of the solution between THC-rich/CBD-poor and THC-poor/CBD-rich cannabis is seen in Fig. 1.

CBD and THC concentrations of confiscated cannabis samples

In a first step, the data was simply examined on a scatterplot showing the concentration pairs of total THC and total CBD. This allowed the observation of three quite distinctive groups, which could be classified by means of the ratio of total THC/total CBD. Samples with a ratio ≥ 3 were classified as THC-rich/CBD-poor cannabis, whereas samples with a ratio ≤ 0.33

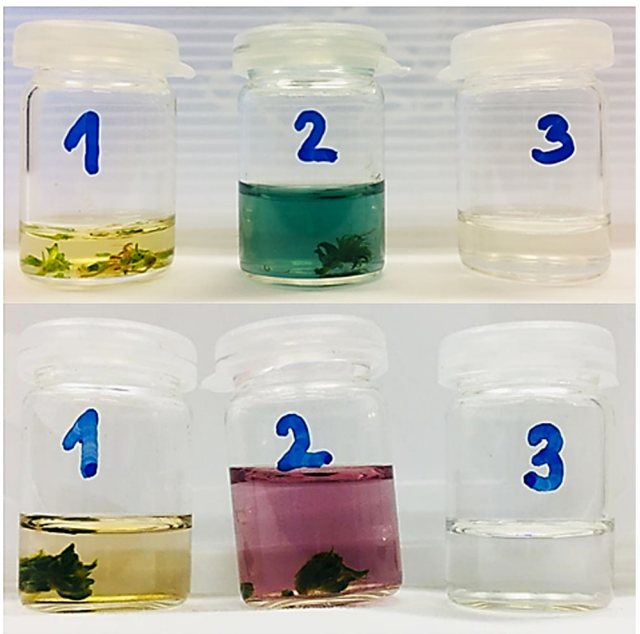

Fig. 1 Colorimetric on-site test (vial 1: cannabis sample with solution 1; vial 2: cannabis sample with solutions 1 + 2; vial 3: solution 1 as reference). Blue coloration (center top) indicates THC-rich cannabis; pink coloration (center bottom) indicates CBD-rich cannabis

were defined as THC-poor/CBD-rich cannabis. The majority of the confiscated samples ($n = 294$, 55%) could be classified as THC-rich/CBD-poor cannabis, whereas 39% of the samples ($n = 205$) were categorized as THC-poor/CBD-rich cannabis. A small number of samples ($n = 32$, 6%) showed a "mixed" cannabinoid profile with a total THC/total CBD ratio between 0.33 and 3 (Fig. 2).

When comparing the intragroup variability (Fig. 3), it can be noticed that the total THC concentrations of the confiscated THC-rich/CBD-poor cannabis samples ranged from 6.7 to 13.0% (interquartile range), with a median of 10.3%. Among the CBD-rich cannabis samples were flowers with up to 24.5% total CBD (25% CBD-acid, 2.5% free CBD, 0.66% total THC), the median concentration, however, is 8.5%. The total THC concentrations in this group are between 0 and 1.7%, with a median value of 0.3% and an interquartile range of 0.2 to 0.5%, concluding that the majority of the CBD-rich samples were below 1% in total THC, and thus legal according to the Swiss law (Fig. 3).

A short Internet research concerning the availability of CBD-cannabis on the Swiss market (Top 10 Google results with keywords "CBD hemp online") revealed that the median CBD concentration is 17%, with an interquartile range of 14.4 to 20.6%, and a maximum of 29.5%. The THC concentrations vary between 0.6 and 0.9% (interquartile range) and show a median of 0.7%.

Determination of cannabinoid concentrations in the CBD-rich cannabis flowers used in the smoking study

The CBD-rich cannabis flowers used in the smoking experiments were analyzed quantitatively for the major cannabinoids in duplicate by HPLC (Fig. 4, Table 1).

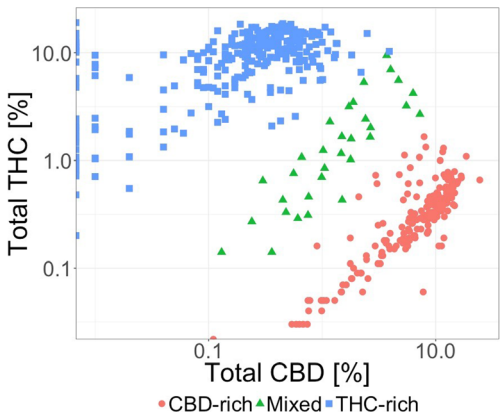

Fig. 2 Distribution of confiscated cannabis samples according to their total THC and CBD concentrations (logarithmic axis)

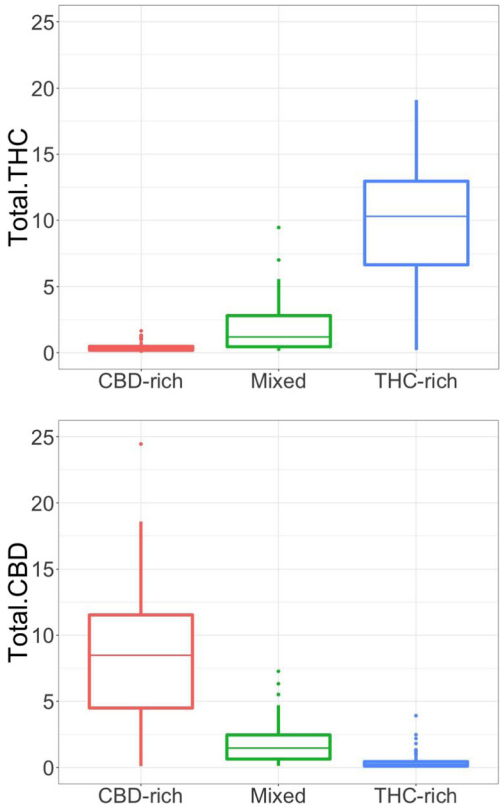

Fig. 3 Box plots showing the intragroup variability of the total THC and total CBD concentrations

Cannabinoid concentrations found in urine and whole blood after smoking CBD-rich cannabis

The applied immunochemical assays yielded negative results for all urine samples collected throughout the study. Quantitative LC-MS/MS analysis confirmed that urinary THCCOOH concentrations were below the LLOQ and thereby clearly below the 50 μg/L cut-off of the immunochemical assays (Table 2). Total CBD, THC, 11-OH-THC, and THCCOOH concentrations in urine were in the range of 7.87–148.79 μg/L, 0–1.34 μg/L, < 1–6.70 μg/L, and < 5–15.71 μg/L, respectively (Table 2). Whole blood cannabinoid concentrations are detailed in Table 3.

Free CBD blood concentrations reached a maximum of 23.4 μg/L after smoking one cigarette, 47.6 μg/L after smoking four cigarettes within 1 h, and 105 μg/L after smoking four cigarettes within 30 min. THC blood concentrations reached a maximum of 1.4 μg/L, 2.1 μg/L, and 6.8 μg/L, respectively. 11-OH-THC was undetectable in all samples (LOD = 0.5 μg/L), and total THCCOOH was only detectable in one sample of session B, but below the LLOQ (< 5 μg/L). Total CBD concentrations were in the range of 16.8–148.0 μg/L (Table 3).

When evaluating the molar ratios of CBD-glucuronide to free CBD in whole blood, it can be noticed that the ratio changes over time. Immediately after smoking, the ratio is

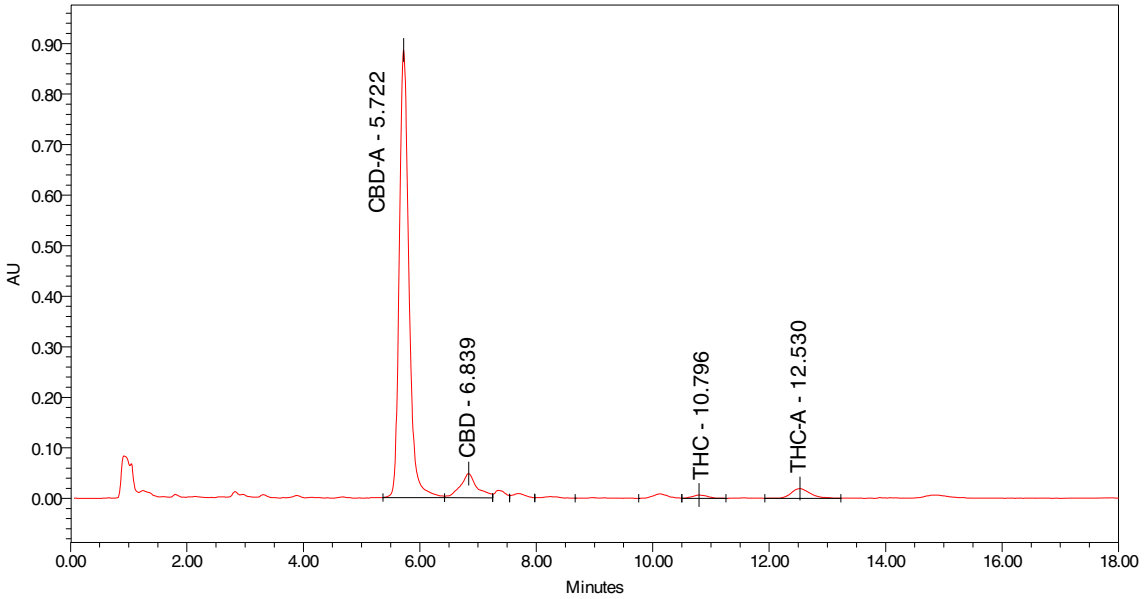

Fig. 4 HPLC-UV chromatogram of a CBD-rich cannabis sample. Detection at 210 nm (AU = absorption units). The compounds elute in the following order: CBD-acid (t_R: 5.72 min), CBD (t_R: 6.84 min), CBN (not detected, expected t_R: 9.02 min), THC (t_R: 10.80 min), THC-acid A (t_R: 12.53 min)

between 0.5 and 1.0, thus mostly in favor of free CBD and up to equality, which is reasonable for the absorption and early distribution phases. As time passes, CBD undergoes glucuronidation and thus the ratio increases (Table 4).

Discussion

Colorimetric on-site test kit and CBD and THC concentrations of confiscated cannabis samples

In Switzerland, the market for legal CBD-rich/THC-poor cannabis expanded rapidly over the past year. According to Swiss Federal Customs Administrations, only five companies were registered at the beginning of 2017, whereas now there are already more than 500 (personal communication). The availability and the consumption of these products are still a widely debated issue in the media and have posed a challenge for public health and especially to the judicial authorities, the police, and forensic experts/toxicologists, as CBD-rich/THC-poor cannabis cannot be differentiated morphologically and/or olfactorily from THC-rich/CBD-poor cannabis. Prior to the development of the new on-site test, the distinction was only possible by laborious analytical methods (mostly GC- or LC-based techniques), which resulted in high financial and administrative expenditures. This pre-test allows the police forces to directly conduct an analysis and take a decision on-site within a very short time and thus to greatly reduce these costs.

Table 1 Cannabinoid concentrations of CBD-rich flowers used in the smoking experiment

CBD	0.45%	THC	0.05%
CBD-acid	9.20%	THC-acid A	0.43%
CBD total	8.52%	THC total	0.43%

Such pre-tests have been successfully applied to distinguish between CBD-rich and THC-rich cannabis samples, whose CBD concentrations are significantly higher than the THC concentrations or vice versa. However, plants with similar concentrations of CBD and THC and therefore a total THC/total CBD ratio between 0.33 and 3 might pose problems when using the pre-test. Experiments with such material ($n = 15$) showed that there is a higher possibility of false negative results (26%). These false negative results indicated a pink coloration after 2 min but changed to a blue coloration after two more minutes, showing the correct result. It is supposed that the test needs more time to stabilize if the THC and CBD concentrations are similar. It is also possible that there is no color change at all, this happened with three samples containing very low concentrations (0.14–0.31% total THC and 0.36–0.75% total CBD). For future use of this pre-test, it is advised to wait at least 4 min prior to reading out the result. With regard to applicability, 1 mL of 4-aminophenol seems to be too little, depending on the sample size. Therefore, it was decided to add solution 1 until the sample was completely immerged.

Even if the coloration of the sample indicates CBD-rich cannabis, this does not automatically imply that it is legal cannabis according to the Swiss narcotics regulations. The total THC concentration needs to be less than 1%, which is verified through quantification of cannabinoids by an HPLC- or GC-based method. In most of the confiscated CBD-rich

Table 2 Urinary cannabinoid concentrations from sessions A–C (creatinine normalized). Total cannabinoid concentrations (free and glucuronidated) were obtained by hydrolysis with β-glucuronidase

	Time (hh:min)	THC total (μg/L)	11-OH-THC total (μg/L)	THCCOOH total (μg/L)	CBD free (μg/L)	CBD total (μg/L)	CBD gluc. (μg/L)
Session A				Cigarette 1			
	01:02	< LLOQ	< LLOQ	< LLOQ	n.d.	36.9	57.5
	12:55	n.d.	< LLOQ	< LLOQ	n.d.	15.4	24.0
Session B				Cigarettes 1–4			
	01:22	1.3	4.8	< LLOQ	1.3	148.8	230.2
	07:44	n.d.	6.7	15.7	< LLOQ	25.0	39.0
	20:00	n.d.	3.3	7.2	n.d.	14.6	22.8
	27:15	n.d.	1.8	5.6	n.d.	7.9	12.3
Session C				Cigarettes 1–4			
	22:22	n.d.	1.2	6.2	n.d.	8.6	13.3

cannabis samples—with a total THC/total CBD ratio < 0.33—the THC concentrations were below this limit. However, 4% of the samples ($n = 8$) showed THC concentrations between 1 and 1.7%, rendering them illegal. Nevertheless, these samples would probably have withstood the on-site test, since the CBD concentrations of all samples were at least 4.5 times higher than their corresponding THC concentrations. For future validation purposes, the minimal concentration ratio between CBD and THC should be determined, for which the test still gives a clear coloration.

Regarding the intragroup variability of the total THC and CBD concentrations in the confiscated cannabis samples, it can be noticed that the median and distribution of total THC concentrations in THC-rich/CBD-poor cannabis is in

Table 3 Whole blood cannabinoid concentrations from sessions A–C. Total cannabinoid concentrations (free and glucuronidated) were obtained by hydrolysis with β-glucuronidase

	Time (hh:min)	THC free (μg/L)	11-OH-THC free (μg/L)	THCCOOH total (μg/L)	CBD free (μg/L)	CBD total (μg/L)	CBD gluc. (μg/L)
Session A				Cigarette 1			
	00:16	1.4	n.d.	n.d.	23.4	35.2	18.4
	00:33	< LLOQ	n.d.	n.d.	18.0	29.7	18.3
	00:47	< LLOQ	n.d.	n.d.	9.8	20.3	16.3
	01:08	n.d.	n.d.	n.d.	6.9	16.8	15.5
Session B	00:00	n.d.	n.d.	n.d.	n.d.	n.d.	
				Cigarette 1			
	00:15	1.3	n.d.	n.d.	21.2	42.6	33.4
				Cigarette 2			
	00:31	2.0	n.d.	n.d.	34.1	85.4	80.0
				Cigarette 3			
	00:47	2.1	n.d.	n.d.	45.4	95.5	78.2
				Cigarette 4			
	01:01	2.0	n.d.	< LLOQ	47.6	122.0	116.1
Session C	00:00	n.d.	n.d.	n.d.	n.d.	n.d.	
				Cigarette 1			
				Cigarette 2			
	00:18	5.9	n.d.	n.d.	64.7	90.2	39.8
				Cigarette 3			
				Cigarette 4			
	00:35	6.8	n.d.	n.d.	105.0	148.0	67.1
	00:51	2.2	n.d.	n.d.	49.4	120.0	110.1

Table 4 Molar ratios of CBD-glucuronide/free CBD in whole blood from sessions A–C

	Time (hh:min)	CBD free (nmol/L)	CBD gluc. (nmol/L)	CBD gluc./CBD free
Session A			Cigarette 1	
	00:16	74.4	37.5	0.5
	00:33	57.2	37.2	0.6
	00:47	31.3	33.3	1.1
	01:08	21.9	31.5	1.4
Session B	00:00	n.d.	n.d.	
			Cigarette 1	
	00:15	67.4	68.0	1.0
			Cigarette 2	
	00:31	108.4	163.1	1.5
			Cigarette 3	
	00:47	144.4	159.3	1.1
			Cigarette 4	
	01:01	151.4	236.6	1.6
Session C	00:00	n.d.	n.d.	
			Cigarette 1	
			Cigarette 2	
	00:18	205.7	81.1	0.4
			Cigarette 3	
			Cigarette 4	
	00:35	333.9	136.7	0.4
	00:51	157.1	224.5	1.4

accordance with data of the THC concentration values 2017 published in the narcotics statistics by the Swiss Society of Forensic Medicine [24].

When comparing the total CBD concentrations of the confiscated CBD-rich/THC-poor cannabis samples with the concentrations reported for CBD-cannabis available on the Swiss market, it becomes apparent that the median concentration offered (17%) is more than twice as high as the measured median concentrations in the seized material. The same applies to the THC concentrations. Even when including a measurement uncertainty, this still does not explain these differences. Some producers maintain a dedicated laboratory for the analysis of their samples, others use external laboratories. Differences in sample preparation (sampling, grinding, extraction method, etc.) certainly contribute to this discrepancy. Therefore, in our opinion, it is important to develop and introduce norms and standards for the analysis of cannabis samples.

Cannabinoid concentrations found in urine and whole blood after smoking CBD-rich cannabis

According to the producer, the cannabis flowers "Alessia," which were used in this study for the smoking experiments, contain between 7 and 17% total CBD. Our analysis showed clear separations of all major cannabinoids and a total CBD concentration of 8.52%, which lies within the producer's declaration, but at the lower end. The wide range of concentration given by the producer not only indicates uncertainty of measurement in the laboratory but probably also variance within the cultivation of the plants and the resultant products themselves. The main concerns, however, are the trace amounts of total THC (in this case 0.43%) present in the legal CBD-rich cannabis. The question arises whether the consumption might cause THC blood levels ≥ 2.2 μg/L (Swiss legal limit 1.5 μg/L, + 30% confidence interval), and thus render the consumer unable to drive from a legal point of view.

Therefore, as a pilot experiment, one volunteer consumed one single and up to four cigarettes of legal CBD-cannabis in a rather short time (less than 1 h), simulating an acute use. In general, the estimation of the CBD and THC dose administered by smoking is very variable. Uncontrollable factors, such as differing efficiency of inhalation and losses by side-stream smoke and pyrolysis, have to be taken into account. It has been demonstrated that the bioavailability of THC ranges between 10 and 50% and that of CBD between 11 and 45%. Furthermore, pyrolytic destruction and side-stream smoke account for losses up to 30 and 50%, respectively [25]. In the present study, the cannabis cigarettes contained 42.7 mg CBD and 2.2 mg THC, which resulted in approximately 29.9 mg CBD and 1.5 mg THC released by smoking and an estimated inhaled dose of 14.9 mg CBD and 0.8 mg THC. Taking into

account the average bioavailability, 4.5 mg CBD and 0.2 mg were systemically available.

Urinary cannabinoid concentrations showed only trace amounts of THC (1.3 μg/L) 1 h after session B (four cigarettes in 1 h). It can be assumed that the urine in session C (four cigarettes in 30 min) would also have been positive if a sample had been taken within 1 h after the end of consumption. In both sessions, no THC was detected anymore after 20 h. Regarding THC metabolites, traces of 11-OH-THC and THCCOOH have been detected in all three sessions, but were only above the LLOQ (1 μg/L and 5 μg/L, respectively) in sessions B and C. However, as revealed by the highly sensitive LC-MS/MS method, all measured THCCOOH concentrations were well below the 50 μg/L cut-off for the applied immunochemical assays, which explains the negative results for all urine samples. The manufacturers do not provide any data on the cross-reactivity with THCOOH-glucuronide. The endogenous metabolite is (−)-*trans*-THCCOOH-glucuronide [26]. Commercially available THCCOOH-glucuronide reference standards are stereochemically different and are therefore not suitable for testing cross-reactivity. However, based on studies with other immunoassays, it can be assumed that cross-reactivity is extensive [27].CBD was predominantly detected as its glucuronide metabolite in all sessions, ranging from 12.3 to 230.2 μg/L. These concentrations were calculated from the difference between total and free CBD and corrected by a factor of 1.56, the quotient between the molar mass of CBD-glucuronide (490.59 g/mol) and CBD (314.46 g/mol). Regarding the free CBD and CBD-glucuronide concentrations, it can be noticed that only in two samples in session B free CBD was detected, of which one was over the LLOQ (1.3 μg/L after 1 h and 22 min). However, it can be assumed that a sample taken at a similar time in session C would also have been positive. Literature on the urinary excretion profile of CBD in humans after smoking is very scarce and has only been reported in one case. It showed that the two major compounds were nonmetabolized CBD (12.1%) and its O-glucuronide (13.3%) [28, 29]. These findings do not correspond to our results, but this may be related to the fact that the subject in this study was chronically treated with daily doses of 600 mg CBD over an unknown period of time. It is also conceivable that sample collection, storage, and processing conditions may be a reason for the differences. Furthermore, a small study has been conducted to investigate the impact of enzymatic and alkaline hydrolysis on CBD concentration in urine. Single intakes of 400 mg CBD showed traces (< LLOQ) of free CBD in urine, but mainly hydrolyzed CBD [30]. A comparison of concentrations, however, remains difficult as CBD was administered orally and the dose was around ten times higher. Other pharmacokinetic studies also showed that CBD, like THC, is subject to a significant first-pass effect; yet, unlike THC, is excreted to a large extent unchanged in the feces [31, 32]. Very recently, the Institute of Forensic Medicine Basel published a similar pilot study with CBD-cannabis [33]. However, in their study, the test person was a nonsmoker and consumed only one or two cigarettes over a 6-h time period. The CBD concentrations in urine after smoking one single CBD cigarette are in good agreement with the results of our pilot study. In addition, we detected THC in two urine samples and the THC metabolites 11-OH-THC and THCCOOH in all samples after smoking multiple CBD cigarettes within 1 h or 30 min.

Analyses of whole blood samples showed detectable THC concentrations in all three smoking sessions. In session C (smoking four cigarettes within 30 min), the THC concentrations exceeded the legal limit for driving under the influence of cannabis according to the Swiss Road traffic regulations. After consumption of two cigarettes within 15 min, the THC concentrations amounted to 5.9 μg/L, respectively to 6.8 μg/L after two additional cigarettes. Both values are clearly over the legal limit of 2.2 μg/L. The concentration dropped to 2.2 μg/L 20 min after smoking the last cigarette, and it can be assumed that it decreased below the legal limit within a few minutes after. It has to be considered that the cannabinoid concentration in the smoked CBD-cannabis flowers is consistent with the median concentration of the confiscated CBD-cannabis samples. However, the reported cannabinoid concentrations of CBD-cannabis available on the Swiss market are highly variable. Therefore, it could be possible to exceed the legal limit already after smoking one cigarette if the THC content of the CBD-rich cannabis and/or the quantities smoked are higher. In addition, other factors like the time taken to smoke a cigarette, puff duration, inhaled smoke volume, and breath-holding after inhalation have to be taken into account [34]. Furthermore, in session B, it can be observed that no considerable accumulation of THC occurs in whole blood. The THC concentrations after the second cigarette remain constant between 1.96 and 2.1 μg/L. This is consistent with observations in pharmacokinetic studies, showing that THC concentrations after smoking decrease by more than 50% within 15 min of reaching the maximum concentration and/or the last inhalation [25, 35–37]. However, accumulation can occur in the fat tissue, as THC is highly lipophilic [32]. It is possible to compare the THC concentrations obtained in this smoking study with concentrations measured in regular smoking studies with THC-rich cannabis, e.g., by Fabritius et al. [37], by comparing the consumed amount of cannabis and the resulting THC concentrations in whole blood. The smoked quantity of THC in the CBD-rich cigarettes was approximately 2.2 mg, compared to about 77 mg in the THC-rich cigarettes in the study by Fabritius et al. [37], which is roughly 3%. Taking into account the factors that can influence the whole blood cannabinoids concentrations (as discussed

above), the measured THC concentrations are fairly consistent and well comparable (1.4 μg/L versus ~40 μg/L after 15 min). Regarding the THC metabolites, 11-OH-THC was undetectable in all samples (LOD = 0.5 μg/L), and THCCOOH was only detectable in one sample of session B, but below the LLOQ.

CBD was detected in its free form and as its glucuronide metabolite in concentrations of 6.9 to 105.0 μg/L and 15.5 to 116.1 μg/L, respectively. Although the metabolism of CBD is well known, very few studies on the blood profile of CBD in humans after smoking are available. Ohlsson et al. conducted a study with deuterium-labeled CBD, which showed mean CBD concentrations in plasma of 32.6 μg/L 15 min after smoking [38]. This is equivalent to a whole blood concentration of 21.8 μg/L, taking into account a plasma/whole blood ratio of 1.5 [35, 39]. Since the quantity of CBD smoked in our study was about twice as high as in the study of Ohlsson et al. [38], our CBD concentration of 23.4 μg/L seems rather low. This discrepancy might be due to interindividual differences in the glucuronidation and elimination rate as well as in the smoking behavior. The recently published pilot study by the Institute of Forensic Medicine Basel also showed higher maximal concentrations of CBD and THC after smoking one cigarette [33]. This can be explained by the faster sampling time (10 min compared to 16 min) after the consumption. Otherwise, the results are in good agreement with our study.

Concerning the molar ratios of CBD-glucuronide to free CBD and the hydrolysis of glucuronide conjugates in general, the degree of glucuronide cleavage should be discussed. In-source fragmentation of the glucuronide conjugates of THC, 11-OH-THC, THCCOOH, and CBD during electrospray ionization leads to the appearance of an additional, earlier eluting peak in the extracted ion chromatogram of the respective unconjugated analyte. Since these chromatographic peaks could not be detected in the extracted ion chromatograms of the hydrolyzed samples, enzymatic hydrolysis of all glucuronide conjugates was assumed to be complete.

Limitations

As interindividual variation in the smoking behavior and the cannabinoid metabolism is very high, the major limitation of this pilot study is that it involved just one participant. This makes the interpretation of results and the comparison with other studies challenging. To overcome these problems, future studies should include a higher number of participants and a longer sampling period. In addition, a test procedure to assess the influence of CBD on fitness to drive should be developed. The current legal situation of THC is based on a "zero tolerance" system using cut-off values. However, in our opinion, it is problematic to make statements about the level of impairment based only on substance concentrations.

Conclusions

Smoking experiments with legal CBD-rich/THC-poor cannabis with one volunteer showed that the legal limit of 2.2 μg/L can be exceeded when smoking multiple cigarettes within a certain time. The consumption of one cigarette only led to a THC concentration of 1.4 μg/L in whole blood. However, various factors, such as interindividual differences in smoking behavior and metabolism or cannabis potency, have to be taken into consideration as well. Therefore, and because possible impairing effects of CBD have not yet been investigated in detail, people should generally be advised to refrain from driving for at least 1 h after smoking CBD-cannabis.

Based on the analysis of a large collective of confiscated cannabis samples, we propose the following classification: plant material with a ratio of total THC/total CBD ≥ 3 is graded as THC-rich/CBD-poor, whereas samples with a ratio ≤ 0.33 are categorized as CBD-rich/THC-poor cannabis. Ninety-four percent of the analyzed samples could be assigned to one of these two categories.

Finally, there is a need for the introduction of norms and standards for the analysis of cannabis samples in the near future, as potency testing and quality control of legal tobacco substitute products will gain more importance. Our analyses showed fairly large discrepancies between the concentrations stated by the producers and those measured in the laboratories. These can be attributed, among other factors, to different sample preparation methods (sampling, grinding, extraction method, etc.). In addition, analyses have shown that the newly introduced on-site test kit for differentiation of CBD- and THC-cannabis is very promising, but needs further validation.

Acknowledgements The authors gratefully acknowledge Alain Broillet, Beatrice Grossen, and Severine Krönert for the evaluation of the on-site test kit and the analyses of confiscated cannabis samples, Thomas Wüthrich for his support with LC-MS/MS data acquisition and the whole team of the Forensic Toxicology laboratory at the Institute of Forensic Medicine Bern for supporting this project.

Compliance with ethical standards

According to the ethics committee of the Canton of Bern, Switzerland, this study does not apply to Art. 3a of the Federal Act on Research involving Human Beings (Human Research Act) since it is a study only involving one participant—who was the principal investigator himself in a self-experiment. Therefore, they did not decide on the application listed with the project ID No. 2018-01037. However, written informed consent was obtained from the participant of the cannabis smoking study.

All procedures performed were in accordance with the ethical standards of the institutional research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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