Excretion of Cannabinoids in Urine after Ingestion of Cannabis Seed Oil

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Abstract

Gas chromatographic–mass spectrometric (GC–MS) quantitation of 25 cannabis sed oils determined Δ^9-tetrahydrocannabinol (THC) concentrations from 3 to 1500 µg/g oil. In a pilot study, the morning urine of six volunteers who had ingested 11 or 22 g of the oil, which contained the highest THC content (1500 µg/g), was collected for six days. The urine samples were screened by immunoassay, and the content of 11-nor-9-carboxy-Δ^9-THC (THCCOOH) was determined by GC–MS. Urine samples were found cannabis positive for up to six days with THCCOOH-equivalent concentrations up to 243 ng/mL by the Abuscreen OnLine immunoassay and THCCOOH contents from 5 to 431 ng/mL by the GC–MS method. All subjects reported THC-specific psychotropic effects.

Introduction

Hemp (*Cannabis sativa* L.) of the fiber type (< 0.5% Δ^9-tetrahydrocannabinol [THC]) or the drug type (> 0.5% THC) (1) can be legally cultivated in Switzerland when used for industrial purposes but not for narcotic production. The Swiss Federal Office of Agriculture even sponsors farmers if they use seeds of controlled origin and of the fiber type. In addition, the products have to be manufactured and marketed by known companies. Stem fibers are used for textiles, ropes, construction material, and paper. The essential oil is added to cosmetics, and the seed fatty oil is processed to pharmaceutical, cosmetic, technical, and food products. Literature data about the THC content of cannabis seeds are rare. Hemphill et al. (2) reported that no cannabinoids were detectable in ungerminated cannabis seeds.

In order to evaluate a possible psychotropic potency of cannabis seed oil, the THC content of 25 oil samples was determined by gas chromatography–mass spectrometry (GC–MS). The oil with the highest THC content was ingested by volunteers, their urine was screened by immunoassay, and the content of 11-nor-9-carboxy-Δ^9-THC (THCCOOH) was determined by GC–MS.

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Experimental

Cannabis seed oil samples

Ten cannabis seed oil samples were obtained from different commercial sources in Switzerland (hemp shops, health food stores, oil manufacturers, farmers). Fifteen oils were produced in our laboratory using cannabis seeds of different types and origins. Cannabis seed oil samples were kept refrigerated at 4°C until analyzed.

Urine samples

Cannabis seed oil (11 g [corresponding to 1 tablespoon or 12 mL] and 22 g) with the highest THC content (1500 µg/g) was administered in the evening as salad oil to six healthy volunteers. Their morning urine was collected over the following six days and analyzed by Abuscreen OnLine (Roche Diagnostic Systems, Branchburg, NJ) and GC–MS. The urine samples were frozen (–24°C) until analyzed.

Analytical procedures

Immunoassay. Urine samples were analyzed by the Abuscreen OnLine assay according to the manufacturer's instructions (3) on a Cobas Mira Plus analyzer (Roche Diagnostic Systems, Basel, Switzerland). A calibration curve ranging from 0 to 150 ng/mL THCCOOH was generated using Abuscreen OnLine cannabinoids calibrators. Samples producing results > 150 ng/mL were diluted 1:1 with twice-distilled water and reanalyzed. The cutoff value was set at 50 ng/mL.

GC–MS. Cannabis seed oil samples were quantitated by the following method: 250 mg oil was spiked with 5 µg THC-d_3 (Radian, Austin, TX) as the internal standard and rigorously shaken with 5 mL of methanol. The oil was separated by freezing (–20°C for 8 h), and 4 mL of the supernatant was evaporated to dryness. The residue was redissolved in 500 µL of *n*-hexane and transferred onto a preconditioned 6-mL SiOH Bond Elut Certify (Varian, Harbor City, CA) solid-phase extraction column. Cannabinoids were eluted with 5 mL of a mixture of *n*-hexane–diethylether (19:1), and 1 µL was injected into the GC–MS. A DB–5MS capillary column (25m × 0.2-mm i.d.;

0.33-µm film thickness, J&W Scientific, Folson, CA), an HP 7673 autosampler, and an HP 5890 series II GC with an HP 5972 mass selective detector were used. The oven temperature was initially held at 70°C for 1.15 min, increased to 120°C at 25°C/min, then to 295°C at 12.5°C/min, and held for 6 min. Splitless injection was used at a temperature of 250°C.

THC peak identification was achieved by the comparison of the retention time (17.30 min) and the relative abundance of four characteristic confirming ions (*m/z* 314, 299, 271, and 231) with the deuterated internal standard (*m/z* 317, 302, 274, and 234).

Quantitation was based on the peak area of a single ion (*m/z* 314) versus the peak area of the corresponding ion of the deuterated internal standard (*m/z* 317). The limit of quantitation was 1 µg/g seed oil. The coefficient of variation at a concentration of 20 µg/g was 6.1% ($n = 6$).

The content of THC acid A in the cannabis seed oils was determined at the same conditions as mentioned above but by online derivatization (Methelute, Pierce, Rockford, IL) at an injector temperature of 225°C. THC acid A reference material was isolated according to (4).

Urine samples were quantitated as follows: 4 mL urine was spiked with 200 ng THCCOOH-d_3 (Research Triangle Institute, Research Triangle Park, NC) as the internal standard. KOH (270 µL, 10M) was added, and the urine was hydrolyzed in a water bath (60°C for 15 min). The urine was then adjusted to pH 4.5 with 700 µL glacial acetic acid and subsequently extracted with a 3-mL C_{18} SPEC microcolumn disc of 15-mg sorbent weight (Ansys, Irvine, CA). The disc was washed with 1 mL diluted acetic acid (20%), dried under vacuum, and transferred into a vial. Acetonitrile (50 µL) and 50 µL bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane were added, and the disc was derivatized at 60°C for 15 min. The disc was dried under a stream of nitrogen and extracted with 100 µL *n*-hexane in an ultrasonic bath. After filtration, a 2-µL aliquot of the extract was injected into a DB-5 MS capillary column (25 m × 0.2-mm i.d.; 0.33-µm film thickness, J&W Scientific). The same instrumentation as mentioned previously was used. The oven temperature was initially held at 200°C for 1 min, increased to 280°C at 15°C/min, and held for 12 min. Splitless injection was performed at a temperature of 260°C.

Identification of THCCOOH was based on the comparison of retention time (16.59 min) and relative abundance of three characteristic confirming ions (*m/z* 371, 473, and 488) with the deuterated internal standard (*m/z* 374, 476, and 491).

Quantitation was based upon the peak area of a single ion (*m/z* 371) versus the peak area of the corresponding ion of the deuterated internal standard (*m/z* 374). The limit of quantitation was 1 ng/mL. The coefficient of variation determined at a concentration of 50 ng/mL was 6.8% ($n = 6$). The cutoff value was set at 15 ng/mL.

Results and Discussion

Cannabis seed oil analysis

The THC content of 25 cannabis seed oil samples analyzed ranged from 3 to 1500 µg/g. There was only one oil with an extraordinarily high THC content. This must have been due to an inappropriate harvesting technique resulting in a contamination of the seeds with the THC-rich leaflets. Of the oils, 88% showed THC levels below the tolerated maximum concentration of 50 µg/g fixed by the Swiss Federal Office of Public Health (5). All 15 oils prepared in our laboratory from fiber-type and from drug-type cannabis seeds had THC contents of 30 µg/g or less, whereas 30% of the commercially available oils had contents above the maximum tolerated value. Seed samples of the cannabis fiber type recommended for cultivation by the Swiss Federal Office of Agriculture showed THC contents of 10 µg/g or less. The amount of THC acid A, the major cannabinoid in fresh-drug-type cannabis plants (1), which was decarboxylated in the GC injector to THC, in the oil samples was less than 10% of the respective THC content.

Psychotropic effects

All six subjects reported THC-specific psychotropic symptoms starting 90 to 150 min after ingestion of cannabis seed oil and lasting 8 to 16 h (subjective qualitative statements). The typical THC effects were time distortion, increased acoustic perception, light confusion, and drowsiness.

Urine analysis

Twelve hours after ingestion of 11 and 22 g cannabis seed oil, which corresponded to doses of 16.5 and 33 mg THC (0.23 to 0.56 mg THC per kg body weight), respectively, all six subjects

Table I. Number of Cannabis-Positive Urine Samples ($n = 6$) After Ingestion of 11g or 22 g of Cannabis Seed Oil (Corresponding to 16.5 mg or 33 mg THC) Determined by Abuscreen OnLine and GC–MS

Time after ingestion (h)	Abuscreen OnLine*	GC–MS†
0	0	0
12	6	6
36	6	6
60	6	6
84	3	5
108	2	2
132	1	2

* Cutoff 50 ng/mL.
† Cutoff 15 ng/mL.

Table II. Urine THCCOOH Concentrations (ng/mL) after Ingestion of 11 g and 22 g of Cannabis Seed Oil as Analyzed by GC–MS*

	Subjects					
Time after ingestion (h)	1	2	3	4	5	6
0	0	0	0	0	0	0
12	298	378	280	81	431	281
36	154	186	121	104	242	263
60	65	71	77	54	57	213
84	35	30	78	10	49	69
108	12	13	31	13	12	46
132	11	9	24	6	5	30

*Subjects 1–3 ingested 22 g cannabis seed oil, and subjects 4–6 ingested 11g cannabis seed oil.

showed positive results with the Abuscreen OnLine immunoassay showing THCCOOH-equivalent levels between 171 and 243 ng/mL urine. After 60 h, all subjects still had concentrations above the cutoff of 50 ng/mL. Creatinine was not determined.

All immunoassay results could be verified by GC–MS with THCCOOH concentrations from 5 to 431 ng/mL. Some negative immunoassay results 84 h and longer after ingestion still showed positive THCCOOH levels with the GC–MS method (Tables I and II). This is in accordance with the findings of other authors (6,7) and is due to the hydrolysis of THCCOOH glucuronides before GC–MS and unknown crossreactivity of these conjugated metabolites with the Abuscreen OnLine reagents.

Neither the immunoassay nor the GC-MS results correlated with the dosage of THC. A 22-g aliquot of cannabis seed oil of 50-µg/g THC (officially tolerated maximum content) would result in an ingestion of 1.1 mg THC. Considering the oral bioavailability of THC of about 5–20% (8,9), 55–220 µg THC would theoretically be absorbed by the body. This dosage certainly would be psychotropically inactive but could possibly produce a cannabinoid positive urine level. Giroud and Rivier (10) found radioimmunoassay-positive THCCOOH urine concentrations (cutoff, 50 ng/mL) after an oral ingestion of 36 g of cannabis seed oil with a THC content of 10 µg/g. The GC–MS and Abuscreen Online levels were below the cutoffs.

Conclusion

We conclude that cannabis seed oil produced from properly collected and processed cannabis seeds contains very low amounts of THC. An oil produced from resin-contaminated seeds may have a THC content high enough to cause typical THC effects and cannabis-positive immunoassay results.

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