Excretion of Cannabinoids in Urine after Ingestion of Cannabis Seed Oil

T. Lehmann 1,*, F. Sager 2, and R. Brenneisen 1
1 Institute of Pharmacy, University of Bern, Baltzerstrasse 5, CH-3012 Bern, Switzerland and 2 Swiss Federal Office of Public Health, Division of Food Science, CH-3097 Liebefeld, Switzerland

Abstract
Gas chromatographic-mass spectrometric (GC-MS) quantitation of 25 cannabis seed oils determined Δ9-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-Δ9-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% Δ9-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-Δ9-THC (THCCOOH) was determined by GC-MS.

* Author to whom correspondence should be addressed.

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 μg oil was spiked with 5 μg THC-d3 (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 (25 m × 0.2-mm i.d.;
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 11 g or 22 g of Cannabis Seed Oil (Corresponding to 16.5 mg or 33 mg THC) Determined by Abuscreen OnLine and GC-MS

<table>
<thead>
<tr>
<th>Time after ingestion (h)</th>
<th>Abuscreen OnLine*</th>
<th>GC-MS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>36</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>84</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>108</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>132</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

* 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*

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

*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.

References


Manuscript received January 13, 1997; revision received March 27, 1997.