

Consumption and Quantitation of Δ^9 -Tetrahydrocannabinol in Commercially Available Hemp Seed Oil Products*

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Abstract

There has been a recent and significant increase in the use and availability of hemp seed oil products. These products are being marketed as a healthy source of essential omega fatty acids when taken orally. Although the health aspects of these oils is open to debate, the probability that oils derived from the hemp seed will contain Δ^9 -tetrahydrocannabinol (THC) is noteworthy. Recent additions to the literature cite a number of studies illustrating that the ingestion of these products results in urinary levels of the THC metabolite, Δ^9 -tetrahydrocannabinol carboxylic acid (THCA), well above the administrative cutoff (50 ng/mL) used during random drug screens. Testing performed by our laboratory found that the concentration of THC in hemp oil products has been reduced considerably since the publication of earlier studies. The purpose of this study is to quantitate the THC levels in commercially available hemp oils and to administer those oils tested to THC-free volunteers to determine urine metabolite levels following several 15-g doses. Two extraction protocols were evaluated for removing THC from the oil matrix: a single step liquid-liquid extraction was compared to a two-phase process using both liquid-liquid and solid-phase techniques. Gas chromatography-mass spectrometry was used to determine THC levels in several products: four from Spectrum Essentials® (3 bottled oils and 1-g capsules), two from Health from the Sun® (1-g capsules and bottled oil) oils, and single samples of both Hempstead® and Hempola® hemp oils. These hemp oil products contained THC concentrations of 36.0, 36.4, 117.5, 79.5, 48.6, 45.7, 21.0, and 11.5 mg/g, respectively. The Abbott AxSYM® FPIA and Roche On-Line® KIMS immunoassays were used to screen the urine samples, and GC-MS was used to determine the amount of THC in each oil as well as confirm and quantitate THCA in the urine of study participants immediately before and 6 h after each dose. Peak THCA levels in the participants' urine ranged from 1 to 49 ng/mL. All volunteers were below positive screen and confirmation cutoffs within 48 h after cessation of ingestion.

* The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Introduction

Δ^9 -Tetrahydrocannabinol (THC) is the psychoactive component in the resin secreted by the flowering buds of the hemp plant, *Cannabis sativa*. Administered most commonly by smoking or ingesting, THC predominantly affects the central nervous (CNS) and cardiovascular systems. Common CNS effects include euphoria, a sense of well being, relaxation, and impaired motor coordination and cognitive ability; hallucinations may result at higher doses. The effects on the cardiovascular system include tachycardia and alterations in blood pressure. Most of these effects are dose related with most symptoms noted from a 20-mg oral or equivalent (one marijuana cigarette with 2% THC) dose (1).

THC is the most widely abused illicit drug in the U.S. today. As a result of this and substantial use of other drugs of abuse, many commercial and federal organizations have instituted workplace drug-testing programs. The effect of a positive drug test can range from not being hired to being fired. In the military a positive drug test may lead to disciplinary action, including felony conviction by court-martial and dishonorable discharge. Consequently, those in the forensic drug-testing industry, and the regulatory agencies that have oversight over them, take very seriously the finding of a drug of abuse in commercial products. Quality-control programs and the use of drug cutoffs to determine positive specimens are employed to reduce the incidence of false accusation resulting from unintended exposure or unintended exposure via legally available products. An example of the effectiveness of this course of action is evidenced by the military increasing the opiate cutoff from 300 ng/mL to 2000 ng/mL, thereby reducing the number of morphine-positive drug tests resulting from poppy seed ingestion. Recently, a similar situation has occurred involving positive drug tests for THC after ingestion of commercially available hemp seed oils (2-6).

The oil of the hemp seed is a good source for many essential

fatty acids (3-, 6-, and 9-omega). Lately, a number of commercial products have come to market touting the benefits of these extracted oils such as reducing the risks of cardiovascular disease and osteoporosis. With increased distribution and use of these products comes a potential problem. Cold-pressing the seeds produces the oil. The source of hemp seeds is from the plant *Cannabis sativa* L., the same plant commonly referred to as marijuana. THC found in this plant is localized primarily in the upper third of the stalk, the leaves, and the flowers (7). Little or no THC is found in the seeds or roots of the plant (8). THC arising in the hemp oils comes from extraction of THC from the leaf and resin material that has adhered to the seeds by the oil as it is produced. This THC contaminant, not surprisingly, finds its way into many of the marketed products. Unintentional administration of THC, by intentional use of hemp seed oils contaminated with THC, will become much more common as the use of these products increases. Consequently, the determination of marijuana usage, which, historically, a positive THC urinalysis implies, will be much more difficult to prove. For example, the Air Force (9) and the Marine Corps (10) recently lost court cases when individuals testing positive for THC argued it was THC-contaminated hemp seed oil, not marijuana use, that caused their positive urinalyses. The drug-testing community therefore must decide whether a significant enough danger exists to warrant a reevaluation of the THC cutoff now in use or, barring this, whether the regulatory agencies should unilaterally require only uncontaminated seeds be used for processing oils. In either case, this is a problem that will not be resolved without proactive choices by the relevant agencies.

The purpose of this study was to determine the concentration of THC in a number of commercially available hemp seed oils. Moreover, it sought to determine the daily urine Δ^9 -tetrahydrocannabinol carboxylic acid (THCA) levels before and after seven consecutive 15-g daily doses. After the last dose of oil, urine samples were collected for one week to determine the length of time an individual remains positive after this dosing regimen.

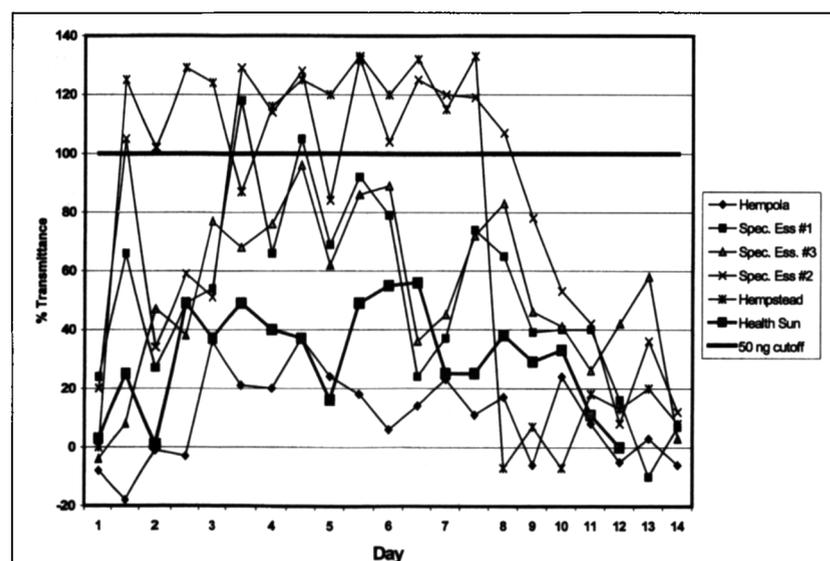


Figure 1. KIMS immunoassays results. The instrument was configured to run in a semiquantitative mode with the HHS/DOD immunoassay positive cutoff of 50 ng/mL set to correspond to a 100% transmittance.

Materials and Methods

Δ^9 -THC and Δ^9 -THC- d_3 were purchased from Radian International (Austin, TX). Hemp oils were purchased from Health from the Sun (Sunapee, NH), Hempstead (Costa Mesa, CA), and Hempola (Port Severn, ON, Canada) and donated by Spectrum Essentials (Petaluma, CA). All solvents, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), ammonium iodide, and dithioerythritol were purchased from Sigma (St. Louis, MO) and were reagent grade.

THC was extracted from hemp oil and analyzed by gas chromatography–mass spectrometry (GC–MS) (11). Two methods of extraction were employed. A four-point (10,000, 5000, 2500, 1000 ng) standard curve was run with each assay. Standards were prepared by adding THC to 50 mg of vegetable oil.

Extraction method 1

Extraction was accomplished by weighing duplicate aliquots of 250 mg, 100 mg, or 50 mg of each hemp oil directly into glass assay tubes. To this 500 or 1000 ng of THC- d_3 (internal standard) was added along with 5 mL of acetonitrile. This mixture was shaken vigorously for 4 h, after which the tubes were removed to a -70°C freezer for 5 min; the centrifuge buckets were chilled along with the oil samples. The samples were removed and centrifuged at 4500 rpm for 10 min. One milliliter of the acetonitrile layer was transferred to a clean tube and dried under nitrogen. The dried samples were then derivatized with 50 mL of MSTFA/ NH_4I /dithioerythritol (1000:2:5, v/w/w) and incubated at 70°C for 20 min. The samples were centrifuged at 3000 rpm for 1 min and transferred directly to GC injection vials; 1 mL was injected for GC–MS analysis.

Extraction method 2

This method was used to quantitate underivatized THC. Duplicate aliquots of 250 mg hemp oil were weighed directly into glass assay tubes. To this 500 or 1000 ng of THC- d_3 (internal standard) was added along with 5 mL of acetonitrile. The specimens were shaken for 4 h after which 4 mL are removed to clean tubes and dried under nitrogen. The resulting residue was dissolved in 1 mL of hexane for subsequent solid-phase extraction (ZTH-CU020, United Chemical Technologies, Bristol, PA). Columns were conditioned using 5 mL of hexane prior to the addition of sample. The sample was allowed to flow via gravity and was washed with 3 mL of hexane before analyte elution with 3 mL of 20% ethyl acetate in hexane. The resulting eluate was dried under nitrogen, the residue reconstituted in 50 mL of 20% ethyl acetate in hexane, and 1 mL injected for GC–MS analysis.

GC–MS analysis was performed using a 5890 GC (Hewlett-Packard, Palo Alto, CA) interfaced to an HP 5972 MS. The GC was equipped with a DB-1MS fused-silica cross-linked methyl silicone, capillary column (15 m \times 0.25-mm i.d., 0.25-mm film thickness, J&W Scientific,

Folsom, CA) with helium (1 mL/min) as the carrier gas. Splitless injections were performed with the GC oven temperature programmed at 150°C (1-min hold), then ramped to 300°C at 50°C/min, and held for 3 min. The injector and MS temperature was 280°C. The MS was operated in the selected ion monitoring (SIM) mode, and ions 303, 315, 318, 386, and 389 were collected (TMS derivative).

Dosing protocol

Volunteers selected to participate in this study were required to submit three pre-study urine samples to verify no recent THC use. The study protocol instructed each subject to collect a urine sample immediately before and 6 h after the dose of oil for seven days. This time period should allow for steady state plasma levels to occur representing the peak in this dosing scheme. This also should represent that time when urine values

are highest. Five volunteers consumed 15 g of hemp oil each morning upon arrival at the laboratory. The 15-g quantity was selected because it approximates one tablespoon, a dose that was frequently reported as being consumed in marijuana-positive urinalysis disciplinary cases involving hemp oils consumption as a defense. One volunteer consumed two 1000-mg Health from the Sun Hemp 1000 gel caps which is the recommended dose indicated on the product. No attempt was made to standardize the subjects with respect to sex, size, body weight, or percent body fat. Urine samples were collected for one week after the last dose of oil to determine an excretion profile and the time when the subjects' urine drops below the screening positive level. Urine THCA concentrations were determined using the standard protocol in use by this laboratory (12,13). In addition to GC-MS analysis, each urine sample was screened using the Abbott AxSYM fluorescence polarization and the Roche On-Line KIMS immunoassay systems using a 50-ng/mL positive cutoff.

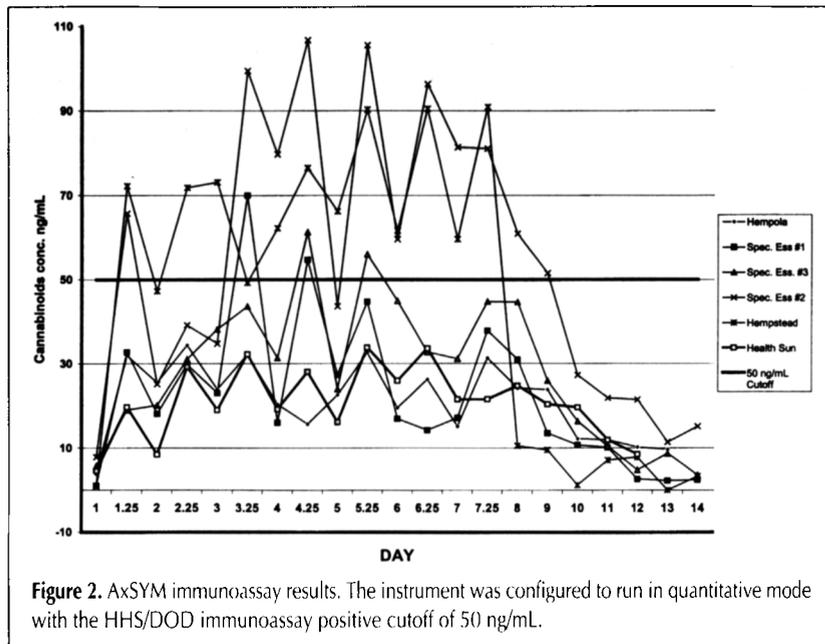


Figure 2. AxSYM immunoassay results. The instrument was configured to run in quantitative mode with the HHS/DOD immunoassay positive cutoff of 50 ng/mL.

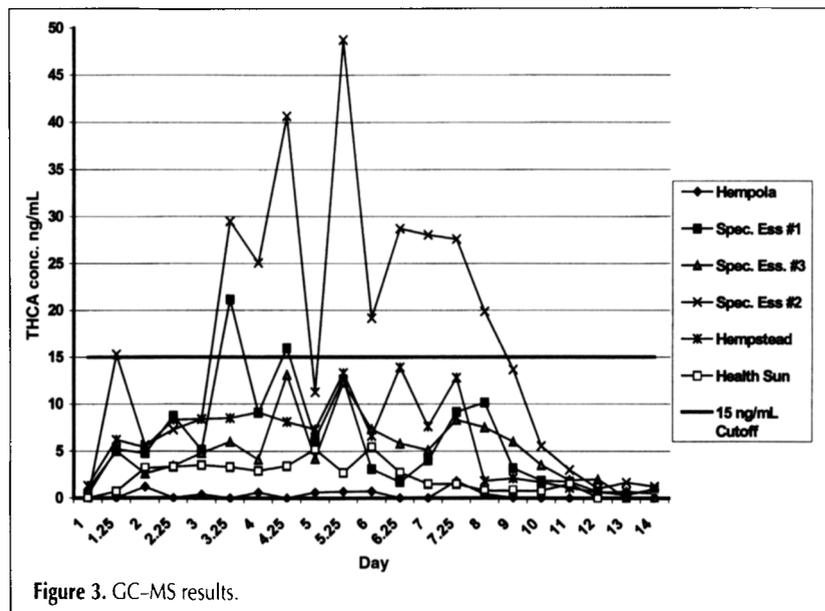


Figure 3. GC-MS results.

Results

Both extraction protocols worked well, given a few caveats. The method in which the THC was derivatized gave the best chromatography but showed a marked reduction in GC-MS response with as much as 70% loss of signal with assays involving more than 20 specimens. The underivatized method had a slightly lower initial GC-MS response but was more stable and reproducible and had good chromatography. Because of its greater stability, the results reported here are from the second extraction method. THC values for the oils tested are as follows: Spectrum Essentials, #1 36.0, #2 117.5, and #3 36.4 mg/g and capsules 79.5 mg/g; Health from the Sun Hemp 1000 1000-mg capsules contained 48.6 mg/g, whereas the bottled oil contained 45.7 mg/g; and the Hempstead and Hempola products contained 21.0 and 11.5 mg/g, respectively.

Urine specimens were screened for cannabinoids with FPIA and KIMS immunoassays using a standard positive cutoff of 50 ng/mL. Similar results were obtained with each immunoassay and are presented in Figures 1 and 2. Subjects ingesting low doses of THC (Health from the Sun "Hemp 1000" capsules and Hempola) had immunoassay results well below the 50-ng/mL immunoassay positive cutoff used by HHS and DOD. Subjects ingesting medium doses of THC in hemp oil (Spectrum Essentials #1 and #3) produced positive immunoassay screen results in the third and fourth days of ingestion. These two subjects had negative immunoassays within 24 h after ingestion ceased. The subject ingesting a high

Table I. Hemp Oil Ingestion and Peak Urine Values*

Product	Concentration ($\mu\text{g THC/g oil}$)	Daily dose ($\mu\text{g THC}$)	Dose per body weight ($\mu\text{g/kg}$)	Peak THCA conc. (ng/mL)
Hempola	11.5	172.5	1.7	1.8
Spectrum Essentials 1	36.0	540.0	7.0	21.1
Spectrum Essentials 2	117.5	1762.5	21.6	48.7
Spectrum Essentials 3	36.4	546.0	9.9	13.1
Hempstead	21.0	315.0	3.6	13.9
Health from the Sun "Hemp 1000" capsules	48.6	97.2	1.3	5.2

* Peak urine levels indicate maximum urine metabolite obtained during study.

dose (Spectrum Essentials #2) screened positive on the first day and was immunoassay negative within 72 h after last ingestion. The subject ingesting the Hempstead hemp oil that had a THC content of 21.0 mg/g oil surprisingly produced positive immunoassay screens after the first dose but never produced a positive above the 15-ng/mL GC-MS cutoff. This individual was immunoassay negative within 24 h after the last ingestion.

Urine THCA levels were determined by GC-MS. Quantitations were achieved by comparing integrated peak areas of the samples with the integrated peak areas of a standard curve. GC-MS quantitations for each subject are presented in Figure 3. Peak urine levels as measured by GC-MS are presented in Table I. GC-MS quantitations paralleled results found with the immunoassays with the low-dose THC consumption producing metabolites at levels well below the 15-ng/mL positive cutoff. Medium doses produced positive GC-MS results in the third and fourth days of consumption. The high-dose oil produced positive GC-MS results in the first day. Of interest is the Hempstead product, which screened positive by both immunoassays but did not produce a positive GC-MS result. Examination of the chromatogram of this specimen indicated that several other cannabinoids that could account for the positive screen were present. All subjects had THCA levels drop below the positive cutoff of 15 ng/mL within 48 h. No psychoactive effects were experienced by any of the subjects during the course of the experiment.

Discussion

This study attempted, using two different extraction techniques, to determine the THC concentration in several commercially available hemp seed oil products. It also sought to determine the urinary THCA concentration in six healthy THC-naïve individuals using both immunoassay and GC-MS techniques.

THC finds its way into the relatively THC-free oil as a contaminant from the THC-rich (leaves and buds) areas of the plant and is transferred to the seed casing during harvesting. Finding THC in hemp oil is not an uncommon occurrence. All of the oils tested contained significant levels of THC. This supports earlier reports that THC was consistently present in commercial and "homemade" hemp oils. The variability seen in

the Spectrum Essentials products is also not uncommon. Lot-to-lot differences, or differences between manufacturers, in THC concentration is most likely the result of poor seed cleaning and preparation or results from a supplier that distributes seeds from plants that inherently contain greater THC levels. Whatever the cause, THC concentrations found in one lot should not be used as any indication of the THC concentrations suspected in other lots.

The ability of hemp oil products to cause a positive result on urinalysis has been reported earlier. The extent to which these products are responsible for causing a THC positive on

random urinalysis is under investigation. If population averages for GI absorption (90–95%), percentage of the dose that is excreted in the urine (20–30%), half-life (24 h), and the manufacturers' dosing recommendations are used to estimate a potential urine value, oils with the lowest levels of THC should be considered as having the potential to cause a positive urinalysis result for THC. Other pertinent information regarding the ability of hemp oil to cause a positive THC result include dose, length of time from last dose until urine sample collection, dosing interval, metabolic capacity, body fat, and urinary output. Many of these parameters are not available to the drug-testing community and, as a result, their effect on an individual's excretion remains unclear. Individual variation, however, should be considered whenever estimates are made regarding urinary excretion of THC metabolite from the ingestion of hemp oil contaminated with THC. Taken collectively, these caveats dictate that positive results from a drug test for THC metabolite be evaluated and scrutinized when considering urinary levels and possible sources of THC.

To date, the scientific community has failed to identify a component of hemp oil not found in marijuana smoke (or vice-versa). Until such a component is identified, the unintentional-use defense will remain a powerful tool used by individuals found positive for THC by urinalysis.

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