Hemp Oil Ingestion Causes Positive Urine Tests for Δ^9-Tetrahydrocannabinol Carboxylic Acid

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Abstract

A hemp oil product (Hemp Liquid Gold™) was purchased from a specialty food store. Fifteen milliliters was consumed by seven adult volunteers. Urine samples were taken from the subjects before ingestion and at 8, 24, and 48 h after the dose was taken. All specimens were screened by enzyme immunoassay with SYVA EMIT II THC 20, THC 50, and THC 100 kits. The tetrahydrocannabinol carboxylic acid (THCA) concentration was determined on all samples by gas chromatography–mass spectrometry (GC–MS) (5). A total of 18 postingestion samples were submitted. Fourteen of the samples screened above the 20-ng cutoff, seven were above the 50-ng cutoff, and two screened greater than the 100-ng cutoff. All of the postingestion samples showed the presence of THCA by GC–MS.

Introduction

Urine tests for drugs of abuse have become an important component of pre-employment testing; evaluation of presumed impairment for truck, bus, and train operators; members of the armed forces; prisoners returning from weekend pass; persons in treatment for chemical dependency; and teenage minor children whose parents are convinced that their child's behavior is caused by drug use. A positive presumptive immunoassay test for phencyclidine, cocaine (benzoylecgonine [BZE]), and marijuana metabolite (tetrahydrocannabinol carboxylic acid [THCA]), if confirmed by gas chromatography–mass spectrometry (GC–MS), is believed to be almost diagnostic of drug abuse. Several years ago, coca tea (Inca tea), allegedly free of cocaine alkaloids, was widely available from health food stores until it was forbidden under federal law because analysis of the tea proved that it contained a small amount of cocaine, which caused a positive test for BZE (1). Confirmed-positive tests for THCA (marijuana) could be caused by ingestion of dronabinol (Marinol™), synthetic Δ^9-tetrahydrocannabinol (THC) in capsules, which are available by prescription. Dronabinol is an effective antiemetic medicine to relieve nausea and vomiting caused by cytotoxic cancer chemotherapy and as an appetite stimulant for persons with the AIDS wasting syndrome. Recently, there have been unconfirmed reports of positive tests for THCA in urine caused by ingestion of marijuana seeds in food such as baked seeded bread (2) or candy (3) (Seedy Sweeties) or by ingestion of hemp oil (4). The present report is, to our knowledge, the first to confirm that ingestion of hemp oil in the recommended dose of 15 mL, will produce a positive test for THC in the urine for 48 h after ingestion.

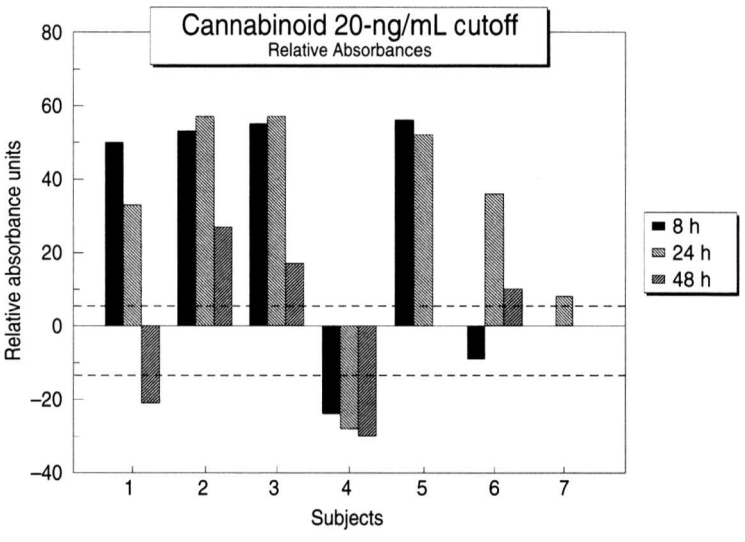

Figure 1. The absorbance of the subjects' samples relative to the cutoff calibrator (0). The relative absorbances for samples taken predose ranged from –46 to –55. The negative control values before and after the patient analyses were –47 and –49. The relative absorbance values for the ± 25% threshold controls were 2 and –19, repectively. The threshold control values are represented by the dashed lines.

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Methods and Materials

Subject protocol

Hemp oil in an 18-oz. plastic bottle (Hemp Liquid Gold™) was purchased in April 1997, from a specialty food store in Northern Virginia. Although the manufacturer has no studies on file for the product, it is said to be rich in 3-omega (2.66 mg/15 mL) and 6-omega alpha linoleic acids and is sold as a homeopathic treatment for certain skin and rheumatic diseases. Seven adult volunteers gave verbal consent for participation in this study. The volunteers were physicians (n = 7), none of whom smoked marijuana or took dronabinol. Each subject was asked to provide a pre-ingestion urine specimen, immediately after which exactly 15 mL of the hemp oil was ingested. None of the subjects experienced symptoms of marijuana intoxication. Urine specimens were requested at 8, 24, and 48-h postingestion. There were no dietary restrictions on the participants. The specimens were refrigerated until completion of the study, at which time they were transported to a Health and Human Services (HHS)-certified reference laboratory for analysis by immunoassay and GC–MS. The hemp oil product was also analyzed for THC content.

Analysis

All specimens were screened by enzyme immunoassay with Syva EMIT II (Behring Diagnostics, San José, CA) THC 20, THC 50, and THC 100 kits. Quality control material at 25% above and below the cutoff of each assay and a drug-free (negative) control were analyzed along with the patient samples. All analyses were performed following the manufacturer's recommended protocol on a Hitachi 747-100 analyzer (Boehringer Mannheim, Indianapolis, IN). The creatinine concentration was determined on each sample by a modified Jaffe reaction. This analysis was also performed on the Hitachi 747.

The THCA concentration was determined on all samples by GC–MS (5). The procedure was the same as that which is used by the laboratory to confirm all initial screening positives. Three milliliters of each patient sample and the calibration and quality control material were extracted by solid-phase extraction (SPE) and prepared as recommended by the manufacturer of the SPE columns (United Chemical Technologies, Bristol, PA). The extracts were derivatized with BSTFA/1%TMCS (Pierce Chemical, Chicago, IL). The extracts were injected onto a 5890II GC equipped with a 12-m HP-1 capillary (0.2-mm i.d.) column and analyzed by a 5970 mass selective detector (MSD) (Hewlett Packard, Palo Alto, CA) The MSD was operated in the selected-ion monitoring (SIM) mode. The ions monitored were 371, 473, and 488 for THCA and 374, 476, and 491 for THCA-d_3. The extracts were quantitated by the internal standard method. THCA-d_3 was the internal standard. A single-point calibration was used. The concentration of the calibrator was at the HHS cutoff of 15 ng/mL. The quality control material included a threshold control (target 15 ng/mL) both before and after the patient samples, a mid-linear range control (target 250 ng/mL), a THCA glucuronide control (target 10 ng/mL), and a drug-free control (negative). The threshold and mid-linear controls were pur-

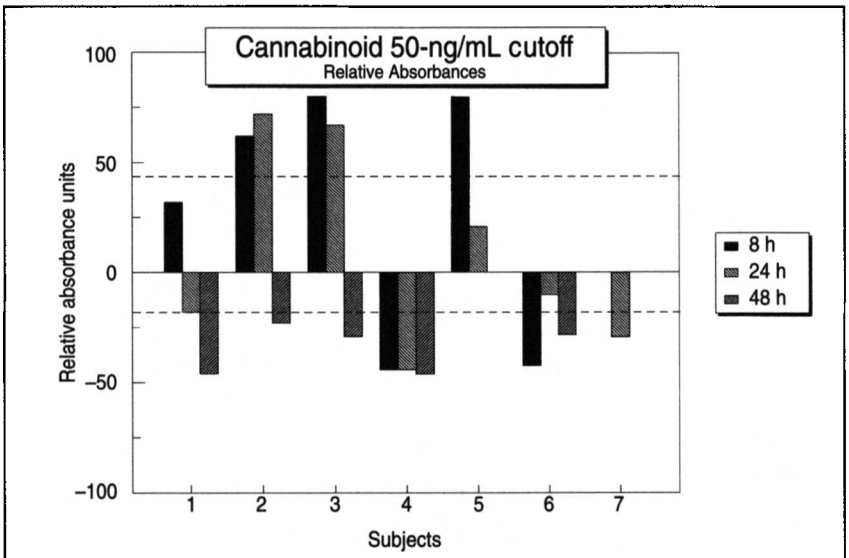

Figure 2. The absorbance of the subjects' samples relative to the cutoff calibrator (0). The relative absorbances for samples taken predose ranged from –51 to –58. The negative control values before and after the patient analyses were –54 and –53. The relative absorbance values for the ± 25% threshold controls were 45 and –17, respectively.

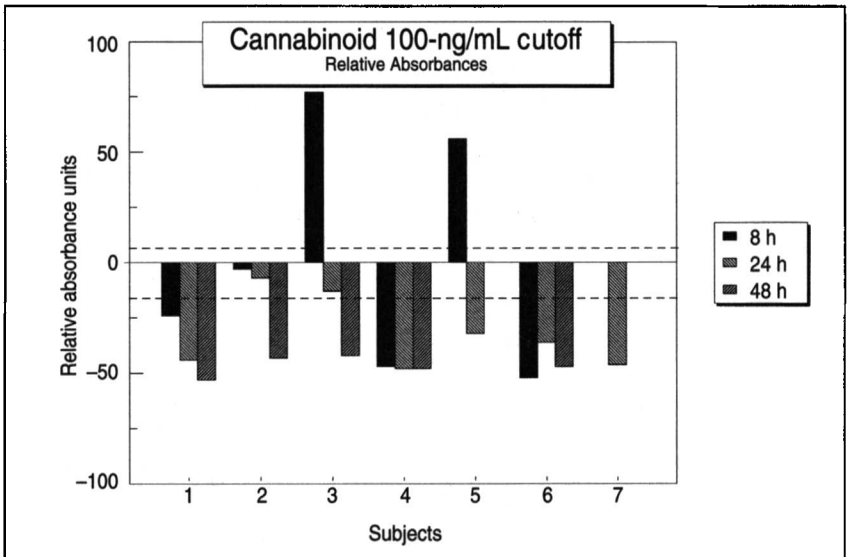

Figure 3. The absorbance of the subjects' samples relative to the cutoff calibrator (0). The relative absorbances taken predose ranged from –50 to –63. The negative control values before and after patient analyses were –58 and –55. The relative absorbance values for the ± 25% threshold controls were 2 and –16, respectively. The threshold controls values are represented by the dashed lines.

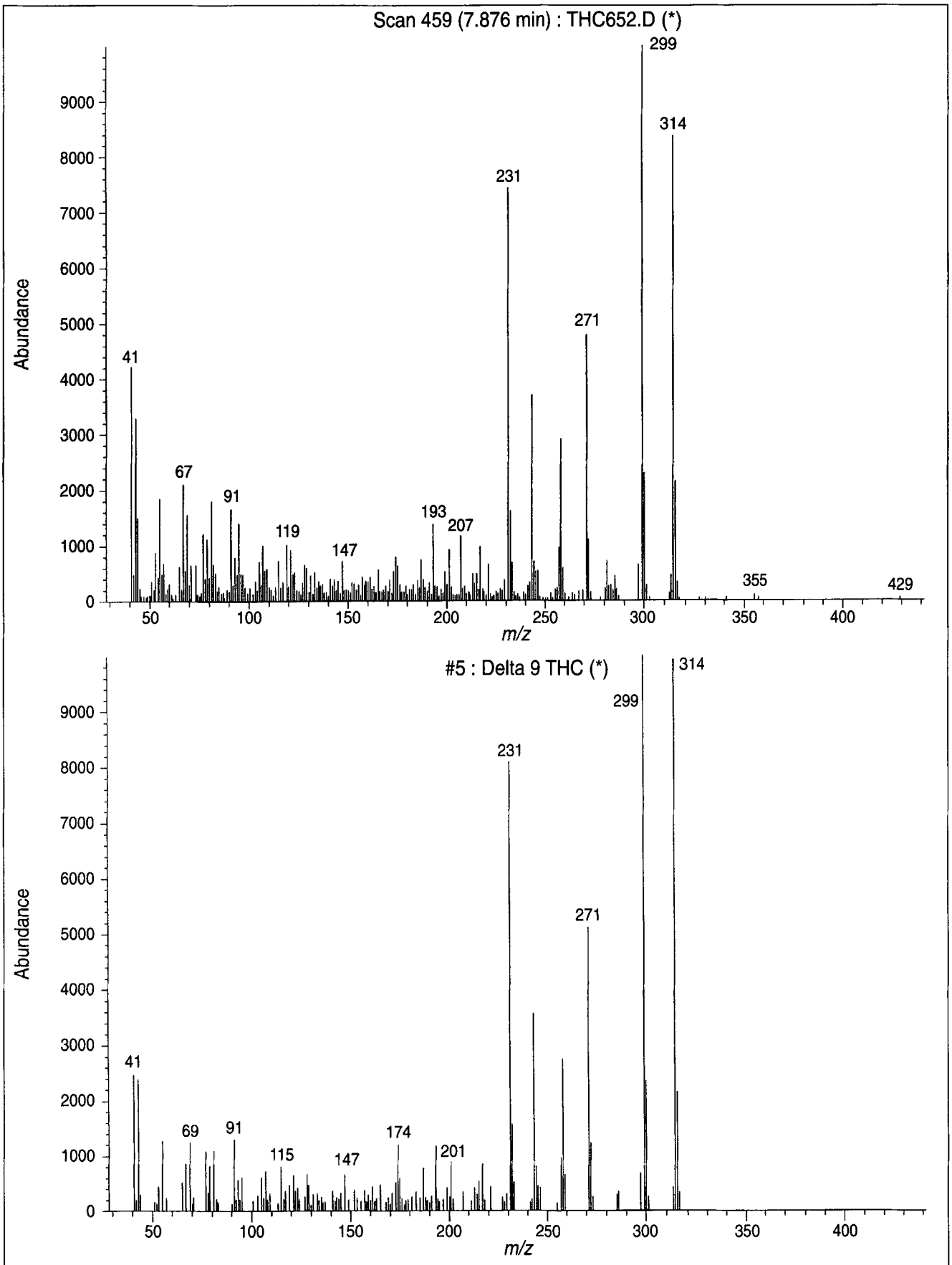

Figure 4. Full scan mass spectra of Hemp Liquid Gold extract compared with the library spectra.

Table I. Urinary THCA Concentrations (ng/mL) Following Ingestion of Hemp Oil

Subject	8 h	24 h	48 h
1	11.0	16.9	7.0
2	22.0	39.2	18.0
3	78.6	34.8	15.0
4	<1.8	3.2	3.2
5	60.7	15.9	NA
6	<1.8	10.6	5.1
7	NA	7.6	NA

* Abbreviations: THCA, tetrahydrocannabinol carboxylic acid; NA, not applicable.

chased from Quality Assurance Service (Augusta, GA). The glucuronide control was prepared inhouse. The stock material was purchased from Alltech Applied Science (State College, PA). The limit of detection and limit of quantitation of the assay was 1.8 ng/mL, and the upper limit of linearity was 500 ng/mL.

The oil was extracted to determine the presence of THC. A portion of the oil was extracted by diluting 1 mL of the hemp oil with 10 mL of acetonitrile. The solution was mixed by rocking for 4 h. The two phases were allowed to separate, and 1 µL of acetonitrile was injected into an HP5890 GC–5970 MSD operated in the full scan mode.

Results

Initial screening

All seven subjects submitted a pre-ingestion urine sample. These samples were negative by EMIT at all three cutoff concentrations. There was no evidence of THCA in any of the specimens by GC–MS. The results of the initial screening tests performed on the postingestion samples are illustrated in Figures 1–3.

Five of the seven subjects submitted three postdose specimens. One subject submitted samples taken at 8- and 24-h postdose only, and another subject submitted just one sample, which was collected at 24-h postdose. Four of the six specimens collected 8-h postdose screened positive at both the 20- and 50-ng/mL cutoffs. Two of these specimens screened positive at the 100-ng/mL cutoff. Of the seven specimens that were collected 24-h postdose, six screened positive at the 20-ng/mL cutoff, three screened positive at the 50-ng cutoff, and none of them screened positive at the 100-ng/mL cutoff. Of the five samples submitted 48-h postdose, three screened positive at the 20-ng cutoff, whereas none were positive at either the 50- or the 100-ng/mL cutoff. All postdose samples submitted from subject 4 screened negative at all three cutoff concentrations. The creatinine values of the 8-, 24-, and 48-h postdose specimens were 94, 52, and 64 mg/dL, respectively.

GC–MS

The range of THCA concentrations for samples submitted from each subject is listed in Table I. All of the postdose samples were positive for THCA by GC–MS. For the six subjects that submitted samples at multiple intervals, the greatest concentrations were found at 8 h in two of the subjects and at 24 h for the other four subjects. All samples submitted by subjects 2, 3, and 5 were ≥ 15 ng/mL. Fourteen of the 18 samples quantitated at greater than 5 ng/mL.

Comparison of the full scan mass spectra of the oil extract to the GC–MSD library showed a match with Δ^9-THC (Figure 4).

Discussion

This study is one of the first to document that hemp oil ingestion can produce a positive test for THCA in urine specimens. THCA was detected for 48 h after a single ingestion of 15 mL, the recommended daily amount. A recent report from Switzerland, where hemp foodstuffs such as hemp tea, hemp noodles, hemp beer, hemp sausage, and hemp oil are available, described four adult patients who were admitted to the hospital after they became intoxicated from hemp oil salad dressing (4). The hemp oil salad dressing contained 0.15% THC, which is within the legal limit for THC content in Swiss foodstuff. THCA was present in the urine and serum specimens from the intoxicated patients.

The U.S. Department of Transportation Guidance on claims of ingestion of hemp food products states that a medical review officer (MRO) must never accept an assertion of consumption of a hemp food product as a basis for verifying a marijuana test as negative (6). When a specimen is found to be positive for THCA, the only legitimate medical explanation for its presence in the Department's drug-testing program is a prescription for dronabinol. This study clearly demonstrated that hemp oil contains Δ^9-THC, and it will cause a positive urine drug screening result for THCA. Federal law specifically excludes oil made from hemp seeds from the definition of marijuana. THC itself is, however, specifically controlled. The legal limit of THC in any consumable product sold in the United States is zero (7).

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