Δ^9-Tetrahydrocannabinol Content of Commercially Available Hemp Products*

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Abstract

Δ^9-Tetrahydrocannabinol (THC) is the main psychoactive compound present in marijuana. THC can also be found, as a contaminant, in some commercially available hemp products marketed in health food stores and on the internet as a good source of essential fatty acids. The products range from oil to alcoholic beverages to nutritional bars to candies, with oil being the most popular and commonly available. The analytical results are separated into two groups, products tested prior to and after publication of 21 CFR Part 1308, "clarification of listing of tetrahydrocannabinols." The data presented are a summary of 79 different hemp products tested for THC. THC was separated by a liquid–liquid or solid–liquid extraction, depending upon the product matrix. THC concentrations range from none detected to 117.5 µg THC/g material. Typical limits of detection for the assay (depending on matrix) are 1.0–2.5 µg THC/g material. Products that were of aqueous base (beer, tea) had much lower limits of detection (2.5 ng/mL). No THC was detected in 58% of the products from group 1 and 86% of the products from group 2. The amounts indicate that THC levels in currently marketed hemp products are significantly lower than in those products available before 2003 and reported in previous studies. The results reported here may be used as a general guideline for the THC content of hemp products recently found in the marketplace today.

Introduction

Δ^9-Tetrahydrocannabinol (THC) is the main psychoactive compound present in marijuana. The primary metabolite monitored by the Department of Defense and the Health and Human Services forensic urine drug testing programs is 11-nor-Δ^9-THC-9-carboxylic acid. Marijuana and hemp, a genotypic variant, are nearly identical members of the genus, *Cannabis sativa*, and differ by the amount of THC produced by each variant (1). Hemp is generally identified as Cannabis strains that produce less than 1% (by weight) of the psychoactive compound THC. Additionally, hemp used for manufacturing or the food industry is legal for import and sale in the U.S. but currently remains illegal to grow. The majority of the hemp used by U.S. industry is grown in Canada under strict government control. Marijuana, on the other hand, is cultivated to contain in excess of 1–20% THC (2–6). THC is found in the oily resin produced and localized mainly in the leaves and flowering buds of the cannabis plant. There is presently no way to distinguish between THC ingested by use of illicit marijuana and that of licit hemp products.

THC affects the central nervous system causing behavioral symptoms which include relaxation, increased awareness of the senses and appetite, and distortion of the environment (7). Physiological effects of THC include increased heart rate, dry mouth and throat, increased appetite, and increased diastolic blood pressure. Absorption of THC is much slower when ingested orally as compared to smoking. This is evidenced by THC detection within seconds in the plasma via the smoking route, whereas plasma THC detection after oral ingestion is not reached until approximately 2 h. Bioavailability of THC through oral ingestion is only 6–18% compared to 18–50% via smoking (7). This difference is caused mainly by THC degradation in the acidic environment of the stomach in addition to first-pass metabolism in the liver. Pharmacokinetically, THC is lipophilic, highly protein bound and exhibits extensive tissue distribution, all leading to a large volume of distribution (8).

Hemp seeds represent the manufacturing starting point for the vast majority of hemp products marketed since the mid-1990s. Hemp seeds are a good source of essential fatty acids, primarily alpha-linolenic acid (omega-3) and linoleic acid (omega-6). They are also found in fish, flaxseed, rapeseed oil, pumpkin seeds, and sunflower seeds. Essential fatty acids (EFA) are necessary fats that humans cannot synthesize, so they must be obtained through diet. EFAs support the cardiovascular, reproductive, immune, and nervous systems. The human body needs EFAs to manufacture and repair cell membranes, enabling the cells to obtain optimum nutrition and expel harmful waste products (9). THC found in manufactured prod-

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ucts is present via contamination from resin produced in the leaves and buds that come into contact with the seed shell. Seed decontamination and manufacturing processes including wash steps and cold pressing for hemp products have improved since the mid-1990s, leading to the much lower THC concentrations currently found in today's commercial products.

The presence of THC in these products has been a source of concern for the military and other workplace drug-testing programs. Ingestion of hemp products has been historically used as a defense in military and civilian trials for many years and continues today despite decreased concentrations of THC in hemp products (10–12). The Division of Forensic Toxicology, Armed Forces Institute of Pathology is often asked to analyze hemp products to determine their THC content in addition to rendering an opinion as to whether or not this THC concentration could be a reasonable cause for a positive THC metabolite urine analysis result. Over the past several years, the laboratory has analyzed 79 different products; the following is a summary of these results.

Experimental

Chemicals and reagents

All solvents were high-performance liquid chromatography (HPLC)-grade and purchased from Fisher Scientific (Pittsburgh, PA). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% TMCS was purchased from Aldrich (Milwaukee, WI). Methanolic standards of THC and THC-d_3 were purchased from Cerilliant (Round Rock, TX).

Sample preparation and extraction

Hemp products were analyzed by a liquid–liquid (oil) or solid–liquid (seeds) extraction depending upon the matrix of the product. The amount of product extracted varied depending on the material: 250 mg for oil and 500–1000 mg for other products. A matrix match standard curve was used for all quantitative assays. For example, flax seed oil was used as the matrix for hemp oil and seed products, and a granola bar was used for hemp bar analysis. Four milliliters of 0.2 N methanolic potassium hydroxide (KOH) was added to the sample. This was followed by washing with hexane four separate times, with the upper organic layer discarded to waste. For solid hemp products, a homogenization step was added after the addition of the methanolic KOH by using a Polytron homogenizer (Brinkmann, Littau, Switzerland). The final wash with hexane included the addition of 1 mL deionized water to reduce methanol's limited miscibility with hexane. Water reduces the miscibility and forces the hexane out of the methanolic solution. The top organic layer was discarded and the solution is made acidic with 1.5 mL of 1 M hydrochloric acid (HCl). THC was then extracted into 3 mL of 10% ethyl acetate in hexane. The solution was mixed and centrifuged for 5 min. The upper organic layer was transferred to clean tubes and evaporated to dryness under nitrogen at 55°C. Samples were derivatized using 50 µL of BSTFA at 70°C for 20 min. Samples were removed from heat, allowed to cool, and transferred to properly labeled gas chromatography–mass spectrometry (GC–MS) vials, and capped.

Instrumental analysis

GC–MS analysis was performed using either an Agilent (Palo Alto, CA) 5890 or 6890 GC coupled to a 5972 or 5973 MS. The GC column was a J&W DB-5MS (20 m × 0.18-mm i.d. × 0.18 µm, Rancho Cordova, CA) with helium as the carrier gas maintained at a constant flow of 1.0 mL/min. One-microliter split injections were made with the injector temperature held at 250°C using a 4-mm inlet liner with deactivated glass wool. An

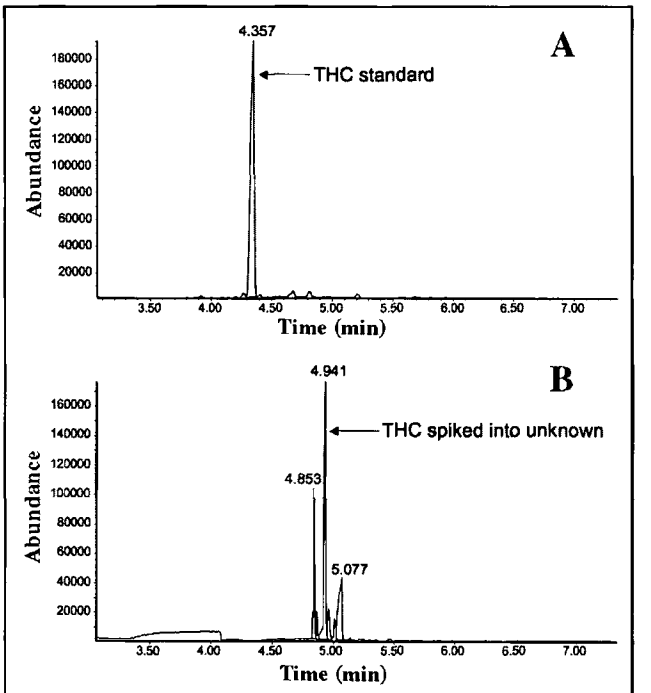

Figure 1. THC spiked in hand lotion for matrix matched standard curve (A), and THC spiked in a hemp tanning lotion (B).

Table I. Types of Hemp Products Tested

Type of Product	Number Tested
Oils	44
Bars	6
Lollipops	6
Seeds	4
Lotions	3
Protein	2
Gummy treats	2
Butters	2
Tablets	2
Teas	2
Beer	1
Chips	1
Cereals	1
Flours	1
Pretzels	1
Vodka	1
Total	**79**

initial GC oven temperature of 160°C was held for 1 min and ramped to 240°C at 40°C/min, from 240°C to 265°C at 10°C/min and then from 265°C to 300°C at 50°C/min with a final hold time of 1 min. The transfer line temperature was set to 280°C. The MS was operated in selected ion monitoring (SIM) acquisition mode. The SIM ions for THC and internal standard (THC-d_3) were (* denotes quantitation ion) *m/z* 303, 371*, 386 for THC and *m/z* 374*, 389 for THC-d_3. The ratio of *m/z* 386/389 was also used for quantitation if interfering matrix peaks from the oils affected peak symmetry of the primary quantitating ions.

Method validation

The linear range of the method was established with each extraction due to the variation in concentrations for each product. A multipoint standard curve with a typical range of 0–50 µg THC/g material was used for quantitation and prepared in a similar matrix. Typical limits of detection for the assay (depending on matrix) are 1.0–2.5 µg THC/g material. Products that were aqueous based (beer, tea) had much lower limits of detection (2.5 ng/mL). The various hemp products were tested as submitted and were analyzed with and without the addition of 10 µg of THC to each sample preparation. THC was added to the samples to ensure extraction efficiency within a given matrix and had acceptance criteria of ± 20%. Retention time shifts are also observed during analysis of the different matrices. The addition of THC to the unknown accounts for the retention time shifts that are sometimes outside of the generally accepted range of ± 2% (Figure 1). By addition of THC, it allows the shift to be monitored for each matrix and allow for accurate quantitation of the unknowns.

Results and Discussion

Table I shows a breakdown of the different types of products tested. Hemp products have expanded from mainly oil to many different products since the mid-1990s. The range of products includes several different beverages, nutritional bars, snacks, and candies. A possible cause for the wide range of available products could be to increase market share by providing hemp products for traditionally non-hemp foods. Another intent appears evident from provocative internet marketing, namely creating legal products that give the impression of illegality for a rebellious younger generation. This strategy, by advertising products that "contain marijuana" or are marijuana flavored, may aim to soften or blur the perception of marijuana as an illegal drug, making it more socially acceptable to market hemp products.

The results of THC analyses are listed in Tables II and III.

Table II. THC Content of Hemp Products Tested Prior to April 21, 2003

Manufacturer	Type	Date Tested	Results (µg/g)
Spectrum Essentials	Oil	20-Mar-98	36.0
Spectrum Essentials	Oil	20-Mar-98	117.5
Spectrum Essentials	Oil	20-Mar-98	36.4
Hempola	Oil	20-Mar-98	11.5
Hempstead	Oil	20-Mar-98	21.0
Health from the Sun	Capsule	20-Mar-98	48.6
Jones Juice Dave	Tea	28-Jul-01	< LOQ (5 ng/mL)
Spectrum Essentials	Oil	29-Aug-01	19
Govinda	Hemp Bar	1-Nov-01	< LOQ (1 µg/g)
Hempola	Oil	11-Dec-01	< LOQ (2.5 µg/g)
Manitoba Harvest	Oil	11-Dec-01	< LOQ (2.5 µg/g)
Manitoba Harvest	Seed	15-Jan-02	< LOQ (1 µg/g)
Hempola	Oil	25-Jan-02	< LOQ (2.5 µg/g)
Spectrum Essentials	Oil	25-Jan-02	23
Spectrum Essentials	Oil	25-Jan-02	29.5
Hempola	Flour	28-Jan-02	< LOQ (1 µg/g)
Spectrum Essentials	Oil	19-Feb-02	2.9
Spectrum Essentials	Oil	19-Feb-02	19.3
Cannabia	Beer	12-Apr-02	ND
Spectrum Essentials	Capsule	12-Apr-02	4.8
Spectrum Essentials	Capsule	12-Apr-02	22.8
Mum's	Oil	11-Jul-02	7.6
Unlabeled	Capsule	11-Jul-02	3.5
Hempola	Oil	11-Jul-02	13
Spectrum Essentials	Capsule	11-Jul-02	68.5
HempNut	Hemp Bar	22-Jul-02	ND
HempStead	Oil	7-Aug-02	12.6
Manitoba Harvest	Oil	7-Aug-02	< LOQ (2.5 µg/g)
Spectrum Essentials	Capsule	9-Aug-02	8.1
Hempola	Oil	14-Aug-02	4.9
Spectrum Essentials	Oil	14-Aug-02	< LOQ (2.5 µg/g)
HempNut	Oil	19-Sep-02	90.4
Orphee	Oil	19-Sep-02	< LOQ (2.5 µg/g)
Hemp Oil Capsules	Capsule	29-Nov-02	< LOQ (2.5 µg/g)
Spectrum Essentials	Capsule	29-Nov-02	5.2
Nutiva Hemp Seed	Bar	30-Dec-02	ND
Nutiva Hemp and Date	Bar	30-Dec-02	ND
Appalachian Blue Ridge	Cereal	30-Dec-02	ND
Spectrum Essentials	Oil	16-Jan-03	ND
Spectrum Essentials	Oil	16-Jan-03	ND
HempNut	Seed	25-Feb-03	< LOQ (1 µg/g)
HempNut	Butter	25-Feb-03	< LOQ (1 µg/g)
Manitoba	Butter	25-Feb-03	ND
Hempzel	Pretzel	26-Feb-03	ND
Ruth's	Chips	18-Mar-03	ND
Hempola	Oil	18-Mar-03	< LOQ (2.5 µg/g)
Hempola	Oil	18-Mar-03	15.2
Manitoba Harvest	Oil	25-Mar-03	ND
Manitoba Harvest	Oil	25-Mar-03	ND
HempNut	Oil	2-Apr-03	6.9

Table III. THC Content of Hemp Products Tested Post April 21, 2003

Manufacturer	Type	Date Tested	Results (µg/g)
Min Tong Company	Tablets	26-Jun-03	ND
Nutiva	Oil	29-Jul-03	7.8
Nutiva	Bar	28-Oct-03	ND
HempNut	Bar	28-Oct-03	ND
Living Harvest	Oil	28-Oct-03	ND
Nutiva	Oil	6-Feb-04	7.4
Chronic Candy	Lollipops	11-Mar-04	ND
Chronic Candy	Gummys	12-Mar-04	ND
Unlabeled	Seed	8-Jul-04	ND
Prairie Emerald Oil	Capsule	8-Jul-04	ND
Hempz Suntan Lotion	Lotion	19-Jul-04	ND
Hempola	Oil	30-Jul-04	ND
Viridian	Oil	30-Jul-04	7.5
Lor Special Drinks	Vodka	27-Aug-04	ND
Swiss Cannabis	Tea	8-Sep-04	ND
Manitoba Harvest	Protein	4-Feb-05	ND
Zand Herbal Formulas	Tablets	4-Feb-05	ND
Nutiva	Oil	10-May-05	ND
Nutiva	Oil	10-May-05	ND
Kiss My Face Corp.	Lotion	13-Jun-05	ND
Emerald Bay	Lotion	3-Feb-06	ND
Chronic Candy	Gummys	10-Jan-07	ND
Chronic Candy	Lollipop	10-Jan-07	1.04
Chronic Candy	Lollipop	10-Jan-07	< LOQ (1 µg/g)
Chronic Candy	Lollipop	10-Jan-07	< LOQ (1 µg/g)
Chronic Candy	Lollipop	10-Jan-07	< LOQ (1 µg/g)
Chronic Candy	Lollipop	10-Jan-07	< LOQ (1 µg/g)
Manitoba Harvest	Seed	1-Mar-07	ND
Nutiva	Protein	13-Jun-07	ND

Table II represents products tested prior to the publication of the Drug Enforcement Agency and Department of Justice's *Federal Register* 21 CFR Part 1308, "clarification of listing of tetrahydrocannabinols." This ruling stated that both natural and synthetic THC be listed as Schedule I drugs of the Controlled Substances Act (13). Table III represents products tested post 21 CFR Part 1308. Product manufacturer is listed as well as type of product tested.

Several other products were tested and excluded from the list for several reasons: they were adulterated (discolored, presence of particulate matter), unlabeled, or not commercially available. Two such products were candy bars manufactured by Tainted Truffles. These products, labeled as "Buddafinga" and "Stoners", contained significant amounts of THC (496 and 360 µg/g, respectively) (14). Other products excluded from the study range from oil received unsealed with leaf material present to homemade bread made with marijuana.

Results of the hemp products tested indicate the amount of THC present in commercially available products is significantly less in products available today than those reported in the past (15–22). As a result, the probability that these products will produce urine THC metabolite levels greater than the DoD and HHS confirmation cutoff of 15 ng/mL is significantly reduced and should not be considered as a realistic cause for a positive urine analysis result.

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