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Cannabinoid Concentrations in Hair from Documented Cannabis Users

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Abstract

Fifty-three head hair specimens were collected from 38 males with a history of cannabis use documented by questionnaire, urinalysis and controlled, double blind administration of Δ^9-tetrahydrocannabinol (THC) in an institutional review board approved protocol. The subjects completed a questionnaire indicating daily cannabis use (N = 18) or non-daily use, i.e. 1 to 5 cannabis cigarettes per week, (N = 20). Drug use was also documented by a positive cannabinoid urinalysis, a hair specimen was collected from each subject and they were admitted to a closed research unit. Additional hair specimens were collected following smoking of two 2.7% THC cigarettes (N = 13) or multiple oral doses totaling 116 mg THC (N = 2). Cannabinoid concentrations in all hair specimens were determined by ELISA and GCMSMS. Pre- and post dose detection rates did not differ statistically, therefore, all 53 specimens were considered as one group for further comparisons. Nineteen specimens (36%) had no detectable THC or 11-nor-9-carboxy-THC (THCCOOH) at the GCMSMS limits of quantification (LOQ) of 1.0 and 0.1 pg/mg hair, respectively. Two specimens (3.8%) had measurable THC only, 14 (26%) THCCOOH only, and 18 (34%) both cannabinoids. Detection rates were significantly different ($p < 0.05$, Fishers' exact test) between daily cannabis users (85%) and non-daily users (52%). There was no difference in detection rates between African American and Caucasian subjects ($p > 0.3$, Fisher's exact test). For specimens with detectable cannabinoids, concentrations ranged from 3.4 to > 100 pg THC/mg and 0.10 to 7.3 pg THCCOOH/mg hair. THC and THCCOOH concentrations were positively correlated ($r = 0.38$, $p < 0.01$, Pearson's product moment correlation). Using an immunoassay cutoff concentration of 5 pg THC equivalents/mg hair, 83% of specimens that screened positive were confirmed by GCMSMS at a cutoff concentration of 0.1 pg THCCOOH/mg hair.

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1. Introduction

Testing hair for drugs of abuse was introduced over 50 years ago [1]. One advantage of hair testing is its larger window of drug detection as compared to other biological fluids [2]. In addition, many drugs are well preserved in hair, the extreme example being cocaine detected in the hair of a 900-year old mummy [3]. Applications of hair testing include criminal investigations, i.e. verifying drug use history, identifying drug facilitated sexual assault, proving drug use in child custody cases, monitoring abstinence of parolees, drug treatment participants or employees, and documenting *in utero* exposure [4,5]. Workplace programs include hair testing due to the ease of collection, difficulty of adulteration and longer detection times. Guidelines for workplace hair testing have been proposed by the Substance Abuse Mental Health Services Administration in the United States [6]. If implemented, the guidelines will establish an initial test cutoff concentration of 1 pg marijuana metabolites/mg hair. The confirmation cutoff concentration has not been finalized but will be either 0.05 or 0.1 pg 11-nor-9-carboxy-Δ^9-tetrahydrocannbinol (THCCOOH)/mg hair.

An important issue is utilization of sufficiently sensitive and specific analytical procedures to detect and quantify drugs in hair. This is especially relevant for cannabinoids that have low concentrations in hair, i.e. Δ^9-tetrahydrocannbinol (THC) in the range of ng/mg of hair or lower, and THCCOOH with pg/mg concentrations [7,8]. Sensitive immunoassays, gas chromatography mass spectrometry (GCMS) with electron impact ionization, GCMS with negative chemical ionization and gas chromatography tandem mass spectrometry (GCMSMS) methods for detecting cannabinoids in hair have been reported [2,7-12]. Sensitivity and specificity of immunoassays remains an issue since many target THCCOOH, despite higher concentrations of THC in hair [5]. Chromatographic assays are more specific, and are methods of choice for confirmation of cannabinoids in hair. More problematic is achieving the necessary detection limits. Lower limits of quantification improve detection rates, but increase the probability of interferences, requiring that assays be carefully validated.

Another important question to address when interpreting a positive cannabinoid result is what dose of drug or frequency of use is required to produce detectable amounts of cannabinoids in hair. Baumgartner reported that a sensitive RIA detected cannabinoids in individuals who self-reported smoking one to two cannabis cigarettes per month [2]. Dose-response data for hair following cannabis use has not been published, but there are data for other drugs, e.g. codeine and cocaine, documenting increasing concentrations in hair with higher doses [13]. Investigators have reported cannabis detection rates of 12-57% in populations of heroin addicts and other chronic drug users who typically use cannabis in addition to their drug of choice [7,10,14,15]. These reports indicate but do not prove that hair analysis fails to detect some frequent cannabis users.

An issue in workplace hair testing was originally identified as racial bias and with later scientific studies was more appropriately labeled hair color bias [13,16,17]. In employment hearings, African Americans claimed discrimination, purporting that drug use was more easily detected in this population because of higher melanin content in their hair. This putative bias appeared to violate equal treatment requirements in employment programs. Mieczkowski discussed these claims and attributed the higher rate of cocaine positive hair tests in an African American population to greater magnitude and frequency of use [18]. Others refined this issue to one of hair color bias and determined that dark hair accumulates more basic drugs than lighter colored hair. Basic drugs bind with high avidity to eumelanin, which is in higher concentration in dark hair [13,17,19]. These research findings give credence to the concept of hair color bias for some drugs, however, preliminary studies indicate that cannabinoid concentrations are similar in dark and light hair [20].

To our knowledge, there have been no studies of deposition of cannabinoids in human hair following controlled administration of cannabis or THC. Most studies rely on self-reports of cannabis use. In cases where individuals have reasons to hide their drug use, self-report can be unreliable [21]. Reported here are hair test results from a group of self-reported cannabis users whose drug use was documented by urinalysis and for some subjects additional controlled administration of THC. All 53 hair specimens collected were tested by GCMSMS and ELISA.

2. Materials and Methods

Subjects

Thirty-eight male cannabis users, ages 21 to 42 years, participated. They provided written informed consent to take part in controlled oral and smoked drug administration studies evaluating the pharmacokinetics and pharmacodynamics of cannabis. Subjects completed a questionnaire regarding drug use habits and had hair collected before and after drug administration. The National Institute on Drug Abuse Institutional Review Board approved the randomized, double blind, double dummy, placebo-controlled clinical studies and subjects were compensated for participation. Based on the structured questionnaires, 15 subjects were African American (AA) daily cannabis users, three Caucasian (C) daily cannabis users, 15 AA who used cannabis 1 to 5 times per week (non-daily users) and 5 C non-daily users. In addition to self-reports, drug use was documented by admitting only those subjects that had a positive urine test at a cannabinoid immunoassay cutoff concentration of 50 ng/mL. After admission, participants were housed on a secure research unit throughout the study, eliminating access to unauthorized drugs.

Clinical research protocol

Upon admission, a head hair specimen was collected from each subject as described below. Prior to drug administration, subjects donated daily urine specimens until cannabinoids were less than 20 ng/mL by immunoassay, a period of time ranging from 9 to 24 days.

For the smoking protocol, subjects smoked a 2.7% THC cigarette (approximately 24 mg THC). Seven days later they smoked a second 2.7% THC cigarette. Later the same day, the post-drug hair specimens were collected.

For the oral dosing protocol, 2 subjects ingested hemp oils of differing THC concentrations and dronabinol in divided doses at meals over 10 weeks. Doses were 0, 0.39, 0.47, 7.5 (dronabinol, synthetic THC), and 14.8 mg per day for five consecutive days with 10 days between dosing regimens. The two highest doses were in the last month of administration. The total THC administered was 116 mg. Hair specimens were collected at the end of the 10-week period 7-10 days after the last dose was administered.

Hair collection protocol

A lock of hair the size of the diameter of a pencil (about 100 mg) was collected from the posterior vertex of the head. Hair was clipped as close to the scalp as possible, placed in airtight containers and stored at −20° C until analysis. Hair strands were aligned to keep root ends together, but in many cases, hair lengths were short and not easily aligned. For the purposes of this investigation and in accordance with routine hair testing practices, the first 3.9 cm of hair closest to the scalp was analyzed to reveal drug use within the last three months. A total of 53 hair specimens were collected. Hair specimens were randomized and blinded prior to analysis at American Medical Laboratories (currently Quest Diagnostics), Las Vegas, NV.

Chemicals

Methanol (Fisher Scientific, Fair Lawn, NJ, USA) and ethyl acetate (Burdick & Jackson, Muskegon, MI) used for hair washing and extraction were HPLC grade. Phosphate buffer (46 mM, pH 7.0) used for reconstitution of the dried hair extracts prior to immunological testing was prepared from K_2HPO_4, purchased from Fisher Scientific. Sulfuric acid was obtained from Anachemia, (Rouses Point, NY, USA).

One hundred (100) or 1000 μg/mL methanolic solutions of THC and THCCOOH were used to prepare calibrators (Cerilliant, Austin, TX, USA) and quality control samples (Alltech, State College, PA, USA). Internal standards for mass spectrometry were THC-d_3 and THCCOOH-d_3 (Cerilliant). Derivatization of the phenol hydroxyl group was accomplished using N-trimethylsilylimidazole (TMSI) (Pierce, Rockford, IL, USA) and, 1,1,1,3,3,3,-hexafluoro2-propanol (HFIP) (Aldrich, Milwaukee, WI, USA) with pentafluoropropionic anhydride (PFPA) (Pierce) for the carboxyl group.

Instrumentation

The positive pressure manifold utilized in the solid phase extraction component of specimen preparation was a Cerex® SPE Processor (CERA Inc., Baldwin Park, CA, USA). Hair sample extracts were analyzed on a Trace 2000 Series GC (Thermo-Finnigan, San Jose, CA, USA) interfaced with a TSQ 7000 Tandem MS (Thermo-Finnigan) equipped with a CTC A 200 S automatic liquid sampler (CTC Analytic, Zwingen, Switzerland) and a DB-5MS capillary column (15 m, 0.25-mm i.d.) with a 1.0-μm film thickness (J & W Scientific, Folsom, CA, USA).

GCMSMS Analysis for THC and THCCOOH in Hair

The first 3.9 cm of hair from the root was cut into segments between two and five mm in length and mixed to ensure homogeneity. A 20 mg portion of hair was incubated with 2.0 mL of 1 M KH_2PO_4 for 30 minutes at 75°C for the initial wash. The buffer was decanted and discarded. Hair segments were washed three additional times with 1.0 mL deionized water, 1.0 mL methanol and finally 1.0 mL deionized water. All washes were discarded. Internal standards THC-d_3 at 20 pg/mg, and THCCOOH-d_3 at 2.0 pg/mg were added to each calibrator, control and specimen prior to digestion. Digestion of the hair was accomplished by adding 1.0 mL of 1.0 M NaOH and heating at 75°C for 30 minutes, or until hair was liquefied. Extraction of cannabinoids from the hair digest utilized Cerex®Polychrom THC Extraction Columns, a positive pressure manifold and 1:1 hexane: ethyl acetate to elute THC followed by 9:1 hexane: ethyl acetate with 1% glacial acetic acid to elute THCCOOH. Calibration samples contained 5 and 0.5 pg/mg THC and THCCOOH, respectively. Controls in certified negative hair matrix were prepared at 0, 3.0 and 7.0 pg THC/mg hair and 0, 0.3 and 0.7 pg THCCOOH/mg hair. A blind quality control sample at a concentration within the linear range was also included with each analytical batch. The final extract for each analyte was derivatized using TMSI/ethyl acetate for THC and HFIP followed by PFPA for THCCOOH. The THCCOOH extracts were evaporated to dryness and reconstituted with 50 μL of toluene. The derivatized extracts were cooled to ambient temperature and 3μL injected into the GCMSMS.

GCMSMS parameters were as follows: splitless injection; transfer line 300°C, a temperature ramp program from 180 to 278 °C at 30 °C /min, to 282 °C at 5 °C /min, to 300 °C at 35 °C / min holding for 2.5 min; and ionization of THC by positive chemical ionization using ammonia as the reagent gas and argon as the collision induced dissociation (CID) gas. THCCOOH was ionized by negative chemical ionization using methane as the reagent gas and argon as the CID gas. Selective reaction monitoring of precursor ions (in italics) yielded the following product ions: *387.5*:265 and 331 m/z for THC; *390.5*: 268 and 334 m/z for THC-d_3; *620.5:* 383 and 492 m/z for THCCOOH and *623.5*: 386 and 495 m/z for THCCOOH-d_3. Quantification was

accomplished by calculating the area ratio of the higher mass product ion (underlined) of analyte to the respective internal standard ion using a single point calibration at 5 pg/mg for THC and 0.5 pg/mg for THCCOOH. Analytical runs were acceptable only when drug-free controls did not contain drug at or above the LOD and those containing drug had measured concentrations that were ± 30% of expected value. LOQs were 1 pg/mg for THC and 0.1 pg/mg for THCCOOH. The upper limits of linearity were 100 pg THC/mg and 25 pg THCCOOH/mg hair. Measuring concentrations of cannabinoid spiked into drug-free hair and requiring that all acceptance criteria be met determined limits for the assay. Intra-assay coefficients of variation at concentrations of 2, 5 and 10 pg THC/mg hair were 14%, 6.1% and 11% and for 0.2, 0.5 and 1.0 pg THCCOOH/mg hair were 9.5%, 2.4%, and 6.2%, respectively. Inter-assay coefficients of variation of the quality control samples were for 3 and 7 pg THC/mg hair 13% and 14% and for 0.3 and 0.7 pg THCCOOH/mg hair 11% and 9.7%, respectively.

Immunoassay for cannabinoids in hair

The One-Step ELISA (Enzyme-Linked ImmunoSorbent Assay) THC Kit ™from International Diagnostic Systems Corporation (IDS, St. Joseph, MI, USA), is a heterogeneous, competitive enzyme immunoassay originally designed to detect cannabinoids in blood, serum, and urine. It was adapted and validated for the analysis of cannabinoids in human hair. The LOD of the assay was 2 pg THC equivalents/mg and a cutoff concentration of 5 pg THC equivalents/mg hair was used to screen all specimens. Intra- and interassay precisions at the cutoff concentration were 1.7 % and 9.3 %, respectively. Cross-reactivities of structurally similar compounds compared to 100% for THC were 283% for 11-nor-9-carboxy-delta-8-THC, 340% for THCCOOH, 56% for delta-8-THC, 51% for 11-hydroxy-delta-9-THC, 51% for cannabinol and 0.1% for cannabidiol. Cross-reactivities for 32 structurally unrelated drugs were all less than 0.01%.

Immunoassay procedure

The first 3.9 cm of hair from the root was cut into segments between two to five mm in length and mixed to ensure homogeneity. For decontamination of external hair surfaces and removal of gel and shampoo residues, 20 mg hair was incubated with 2.0 mL of methanol for five minutes at ambient temperature with occasional swirling. Solvent was decanted and discarded. Three mL methanol were added to each 20 mg washed hair sample and tubes heated for two hours at 70-75°C. After cooling to ambient temperature, methanol was transferred to a clean, labeled tube and evaporated to dryness under nitrogen at 37°C. Hair extract residues were reconstituted with 600 μL of pH 7.0 phosphate buffer, vortexed and submitted for immunoassay.

Statistical tests

Several procedures for detecting use of cannabis or THC were evaluated. We determined the fraction of specimens that were positive using each of the following criteria: GCMSMS for THC ≥ LOQ, GCMSMS for THCCOOH ≥ LOQ, immunoassay cannabinoids ≥ 5 pg THC equivalents/mg hair, and both immunoassay and THCCOOH ≥ respective cutoff concentrations. Fisher's exact test, two-tailed, was used to compare the fractions of positive specimens, i.e. detection rates, for daily and non-daily users, AA and C subjects, and to test for independence of detection rates for subjects before and after smoked cannabis [22]. Statistics were not separately produced for data collected following oral ingestion since the number of subjects was small.

THC and THCCOOH concentrations were compared using Pearson's product moment correlation. Concentrations equal to or above the LOQ for these cannabinoids were compared between groups using the Mann-Whitney Rank Sum Test. Cannabinoid concentrations before

and after smoking were compared using this test and also a one way analysis of variance (ANOVA) to determine if groups were independent [22].

3. Results

Nineteen (36%) of the 53 hair specimens had no detectable THC or THCCOOH at or above the GCMSMS LOQs, 1.0 and 0.1 pg/mg hair, respectively (see Table 1). THCCOOH is present in more hair specimens than THC with 14 specimens (26%) having only THCCOOH, two (3.8%) only THC and 18 (34%) both. As might be expected, the specimens from daily users were more likely to be positive; 17 of 20 (85%) daily users and 17 of 33 (52%) non-daily users hair specimens have either THC or THCCOOH present (see Table 2). Detection rates using any method for identifying a positive result (Fisher's exact test, $p > 0.2$) and concentrations of either THC or THCCOOH (Mann-Whitney Rank Sum Test, $p > 0.2$; ANOVA, $p > 0.05$) did not differ between the pre and post dose groups. Since the groups were not independent, they were combined for further comparisons. Detection rates for daily users were statistically higher than that of non-daily users regardless of the choice of detection method used in this study, see Table 2 ($p < 0.05$, Fisher's exact test). The fraction of positive specimens for AA subjects was not significantly different from that of C subjects; 26 of 40 (65%) AA and 8 of 13 (62%) C ($p > 0.3$, Fisher's exact test).

The immunoassay with a cutoff concentration of 5 pg THC equivalents/mg hair detected cannabinoids in 43% of specimens (see Table 2). Of the 23 positive specimens, 19 (83%) confirmed for THCCOOH. One of the specimens with a positive screen had detectable THC with no detectable THCCOOH while five had THCCOOH only. Immunoassay cross-reactivity to THCCOOH was much higher than to THC yielding a cutoff concentration of approximately 1.5 pg THCCOOH/mg hair. There was little interference from non-cannabinoid compounds and the intra-assay coefficient of variation for absorbance at the cutoff concentration was less than 2%.

Most forensic programs testing large numbers of specimens for cannabinoids screen them first by immunoassay, then confirm positive screening results by identifying THCCOOH. If one examines the tandem screening and confirmation results and requires both to be positive, i.e. cutoff concentrations of 5 pg THC equivalents/mg and 0.1 pg THCCOOH/mg hair, respectively, 60% of daily cannabis users compared to 24% of non-daily cannabis users were positive for cannabis use. These detection rates were statistically different ($p < 0.05$, Fisher's exact test).

For those specimens with detectable cannabinoids, the range of concentrations for THC was 3.4 to > 100 pg/mg of hair and for THCCOOH 0.10 (the LOQ) to 7.3 pg/mg hair (Table 1). THC and THCCOOH concentrations were positively correlated ($r = 0.38$, $p < 0.01$, Pearson's product moment correlation, excluding the subject with > 100 pg THC/mg hair). Median THC and THCCOOH concentrations were higher for daily users and AA subjects, but elevations were not statistically significant (Mann-Whitney Rank Sum Test, all $p > 0.2$, see Figures 1 and 2).

4. Discussion

Sixty-four percent of hair specimens from cannabis users in this study had detectable THC or THCCOOH using GCMSMS with LOQs of 1 and 0.1 pg/mg hair, respectively. Subjects self-reported drug use habits and their cannabis use was confirmed by a positive urine test for cannabinoids (cutoff concentration 50 ng/mL) prior to collecting hair specimens. To further ensure use of THC, 13 of these subjects smoked two 2.5% THC cannabis cigarettes and two subjects ingested 116 mg of THC in divided daily oral doses over 10 weeks. Hair specimens

were collected after dosing and their cannabinoid concentrations were included with results for the original 38 hair specimens before determining detection rates and other statistical comparisons. Other investigators using methods with THCCOOH LOQs ranging from 0.5 to 10 pg/mg and examining various populations of drug users reported detection rates of 20-57 % [9,10,14]. As expected, detection rate in the present study is higher for daily users (85%) who reported smoking from one to several joints or blunts each day than for non-daily users (52%) who smoked 1-5 joints per week.

Seven of 13 subjects who smoked cannabis in the controlled administration study had a positive hair test for either THC of THCCOOH using GCMSMS. They smoked 48 mg of THC but times of smoking in relation to specimen collection must be noted. Hair was collected one week after subjects smoked the first cigarette and later the same day that the second cigarette was smoked. Cannabinoids from the second cigarette would not have had sufficient time to enter the hair from the blood since typical initial detection times are 7 to 10 days. However, drugs deposited in hair from sweat and/or sebum would have had sufficient time to enter hair after the second cigarette. The two subjects ingesting THC orally took the drug daily over a period of 10 weeks. Daily doses varied from 0, placebo for one week, up to 14.8 mg per day for five days during another week with a total dose of 116 mg THC. Subjects received the highest doses in the last month of administration. Hair specimens were not collected until seven to ten days after the last THC dose. Neither specimen contained measurable THC and only one had THCCOOH at a concentration of 0.1 pg/mg hair. Even though the number of subjects is small, one can observe that THC does not readily accumulate in hair following oral administration.

For many drugs the parent compound is in much higher concentration in hair than water-soluble metabolites and this is also true for cannabinoids. Concentrations ranged from 3.4 to > 100 pg THC/mg hair compared to 0.10 to 7.3 pg THCCOOH/mg hair. THC concentrations compared well with those reported by other investigators [7,8,10], while THCCOOH concentrations were similar to those reported by Moore et al. [9], but lower than the range of 20 to 330 pg/mg hair reported by others [7,14]. In a study of 3,678 hair cannabinoid positive specimens submitted from a general drug testing population, a mean THCCOOH concentration of 0.67 pg/mg was reported [11], but the LOQ of the method was not included. Lower quantification limits will yield lower median and mean concentrations making specification of limits an essential factor in interpreting and comparing results. In the present study, given the testing limits for THC and THCCOOH, measuring THCCOOH identified more drug users, despite its much lower concentration range. THCCOOH was detected in 14 of 53 specimens that had no measurable THC. Only two specimens contained THC without THCCOOH present.

Median THC concentration for AA (34.3 pg/mg) was higher than for C (18.8 pg/mg). This difference was not statistically significant using a nonparametric statistical test, and there was significant overlap in THC concentrations for the two groups (see Figure 1). In fact, the subject with the highest THC concentration was C, i.e. > 100 pg/mg hair. THCCOOH concentrations also did not differ by race (Figure 2). Racial bias has been reported for cocaine in hair, supported by studies that found a higher concentration of cocaine in hair from black males than Caucasian males [16,23]. Additional studies changed the focus from racial to hair color bias when it was documented that basic drugs bind to eumelanin, which is in higher concentration in dark hair [13,17]. Cannabinoids were not among the compounds studied. Smeal, et al., concluded in a recent preliminary study using rats that pigmentation did not appear to be a factor in the incorporation of THC and THCCOOH in hair [20]. Findings in the present study also indicated no difference in detection rates and no statistical difference in THC or THCCOOH concentrations between AA and C subjects.

Most workplace hair testing programs employ screening tests to inexpensively identify negative specimens. Presumptively positive specimens must be confirmed by measuring

THCCOOH with a method based on a scientific principle different from that of the screening test. THCCOOH is confirmed instead of THC because the parent drug may be deposited in hair by external contamination. Results of this study indicate that measurement of THCCOOH will also improve detection rates. The Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, in the United States is currently proposing guidelines for workplace hair testing [6]. The screening test proposed is an immunoassay with a cutoff concentration of 1 pg marijuana metabolites/mg hair. The confirmation cutoff concentration will be either 0.05 or 0.1 pg THCCOOH/mg hair. The screening cutoff concentration is lower than that utilized in this study. Assays with lower screening limits may increase detection rates. The Society of Hair Testing also proposed methods and cutoff concentrations; initial test THC ≥ 100 pg/mg and confirmation THC ≥ 100 pg/mg or THCCOOH ≥ 0.2 pg/mg hair [24]. Comparing initial test proposals is complicated by differences in specificities of immunoassays and cannabinoids used as calibrators. For example, note that for the immunoassay used in the present study the cross-reactivity of THCCOOH is over 3 times higher than that of THC, making the cutoff concentration of 5 pg THC/mg hair approximately comparable to 1.5 pg THCCOOH/mg hair. Confirmation comparisons have fewer variables. The cutoff concentration for THCCOOH in the present study falls between those recommended by the two organizations.

Data also were examined to identify specimens that had a positive screening test (cutoff concentration 5 pg THC equivalents/mg) and a positive confirmation test (cutoff concentration 0.1 pg THCCOOH/mg hair). Sixty percent (60%) of daily users and 24% of non-daily users hair met these criteria. The confirmation rate was 83%. Most current cannabinoid screening tests have a higher cross-reactivity for metabolites than parent drug. One might expect the detection rate to increase if the screening test had a higher cross-reactivity to THC due to its presence in higher concentrations; however, 26% of specimens in this study had THC less than or equal to 1 pg/mg. Also, making the immunoassay more specific for THC may decrease the confirmation rate to an unacceptable level. Manufacturers may be able to change the specificity of the initial test and optimize the detection and confirmation rates, but at present immunoassays similar to that used in this study appear to have acceptable performance.

5. Conclusions

Hair specimens were collected prior to (N = 38) and after controlled administration of two 2.7% THC cigarettes (N =13) and multiple oral doses of THC in hemp oil (total 116 mg THC/subject, N =2) and analyzed by ELISA and GCMSMS. Nineteen specimens (36%) had no detectable THC or THCCOOH at the GCMSMS LOQ of 1.0 and 0.1 pg/mg hair, respectively. Two specimens (3.8%) had THC only, 14 (26%) THCCOOH only, and 18 (34%) both cannabinoids. Following oral ingestion, no THC was detected and one of two hair specimens contained detectable THCCOOH. Cannabis use was detected for 17 of 20 (85%) daily users and 17 of 33 (52%) non-daily users (1-5 cannabis cigarettes/week), a statistically significant difference ($p < 0.05$, Fisher's exact test). There was no difference in detection rates between AA and C subjects ($p > 0.3$, Fisher's exact test). For specimens with detectable cannabinoids, concentrations ranged from 3.4 to > 100 pg THC/mg and from 0.10 to 7.3 pg THCCOOH/mg hair. THC and THCCOOH concentrations were positively correlated ($r = 0.38$, $p < 0.01$, Pearson's product moment correlation). Using an immunoassay cutoff concentration of 5 pg THC equivalents/mg hair, 83% of specimens that screened positive were confirmed by GCMSMS at a cutoff concentration of 0.1 pg THCCOOH/mg hair.

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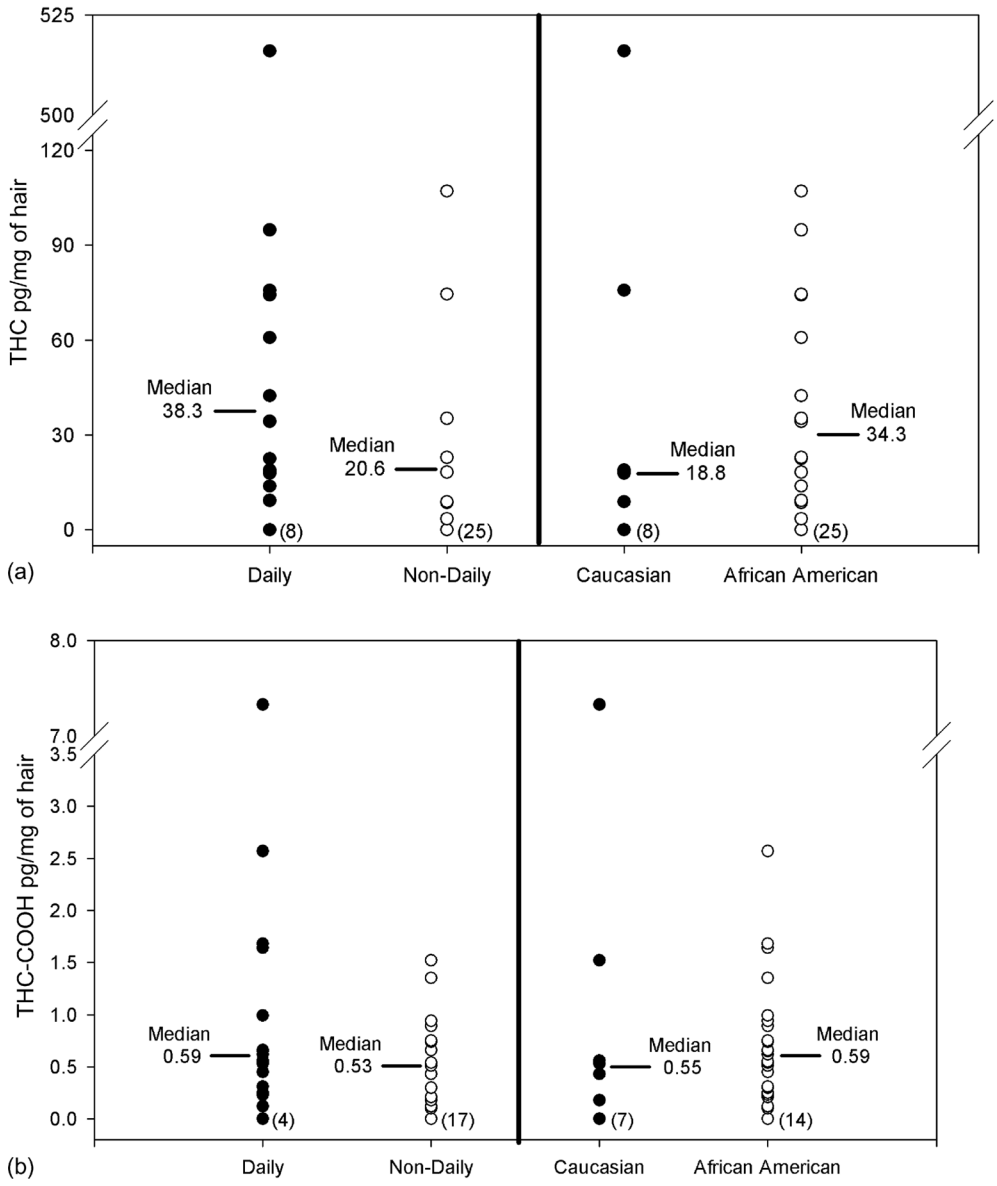

Figure 1.

Concentrations of (a) Δ^9-tetrahydrocannbinol (THC) and (b) 11-nor-Δ^9-tetrahydrocannbinol-9-carboxylic acid (THCCOOH) in hair of cannabis users. There was no statistically significant difference in concentrations of either cannabinoid using the Mann-Whitney Rank Sum Test between daily and non-daily users ($p > 0.2$) or between Caucasians and African Americans ($p > 0.7$). The numbers of specimens with concentrations less than the LOQ are in parentheses and are not included in median calculations.

Table 1

Cannabinoid concentrations in hair from known cannabis users before and after controlled administration of smoked cannabis and oral tetrahydrocannabinol

Specimen	GCMSMS THC pg/mg hair	GCMSMS THCCOOH pg/mg hair	Screen THC[1] cutoff concentration 5 pg THC/mg hair	Frequency Use[2]	Race[3]	Dose[4]
1	0.0	0.99	P	Daily	AA	N
2	95	0.66	P	Daily	AA	N
3	61	0.23	P	Daily	AA	N
4	0.0	0.00	N	Daily	AA	N
5	0.0	0.25	N	Daily	AA	N
6	74	1.6	P	Daily	AA	N
7	0.0	0.55	N	Daily	AA	N
8	0.0	0.31	N	Daily	AA	N
9	0.0	2.6	P	Daily	AA	N
10	9.3	0.12	N	Daily	AA	N
11	14	1.7	P	Daily	AA	N
12	22	0.45	P	Daily	AA	N
13	0.0	0.00	N	Daily	AA	N
14	34	0.62	P	Daily	AA	N
15	0.0	0.00	N	Daily	AA	N
16	42	0.65	P	Daily	AA	S
17	76	0.56	P	Daily	C	N
18	>100	7.3	P	Daily	C	N
19	19	0.00	P	Daily	C	N
20	18	0.53	P	Daily	C	S
21	0.0	0.00	N	Non-Daily	AA	N
22	0.0	0.00	N	Non-Daily	AA	N
23	74	0.94	P	Non-Daily	AA	N
24	0.0	0.00	N	Non-Daily	AA	N
25	0.0	0.00	N	Non-Daily	AA	N
26	3.4	0.54	P	Non-Daily	AA	N
27	110	1.3	N	Non-Daily	AA	N
28	0.0	0.00	N	Non-Daily	AA	N
29	0.0	0.00	N	Non-Daily	AA	N
30	18	0.51	P	Non-Daily	AA	N
31	35	0.21	P	Non-Daily	AA	N
32	8.6	0.10	N	Non-Daily	AA	N
33	0.0	0.12	N	Non-Daily	AA	N
34	0.0	0.74	P	Non-Daily	AA	N
35	0.0	0.00	P	Non-Daily	AA	N
36	23	0.89	P	Non-Daily	AA	S
37	0.0	0.00	N	Non-Daily	AA	S
38	0.0	0.75	P	Non-Daily	AA	S
39	0.0	0.00	P	Non-Daily	AA	S
40	0.0	0.00	N	Non-Daily	AA	S
41	0.0	0.30	N	Non-Daily	AA	S
42	0.0	0.66	N	Non-Daily	AA	S
43	0.0	0.10	N	Non-Daily	AA	O
44	0.0	0.00	N	Non-Daily	AA	O
45	0.0	0.00	N	Non-Daily	C	N
46	0.0	0.00	N	Non-Daily	C	N
47	0.0	0.43	N	Non-Daily	C	N
48	0.0	1.5	P	Non-Daily	C	N
49	8.9	0.00	N	Non-Daily	C	N
50	0.0	0.00	N	Non-Daily	C	S

Specimen	GCMSMS THC pg/mg hair	GCMSMS THCCOOH pg/mg hair	Screen THC[1] cutoff concentration 5 pg THC/mg hair	Frequency Use[2]	Race[3]	Dose[4]
51	0.0	0.00	N	Non-Daily	C	S
52	0.0	0.18	N	Non-Daily	C	S
53	0.0	0.00	N	Non-Daily	C	S

[1] P positive, N negative

[2] Non-daily = 1 to 5 cannabis cigarettes per week

[3] AA African American, C Caucasian

[4] N = none or pre-dose, S = smoked two 24 mg THC cigarettes, O = oral 116 mg THC over 10 weeks

Table 2

Detection rates for known cannabis users with different laboratory methods for designating a positive hair specimen. (Screen cutoff concentrations in pg THC equivalents/mg hair; other cutoff concentrations in pg/mg hair.)

		Percent positive hair specimens .				
	N	**THC ≥ 1**	**THCCOOH ≥ 0.1**	**THC ≥ 1 or THCCOOH ≥ 0.1**	**Screen ≥ 5**	**Screen ≥ 5 & THCCOOH ≥ 0.1**
Daily[1]	20	**60**	**80**	**85**	**65**	**60**
Non-daily[1]	33	**24**	**48**	**52**	**30**	**24**
AA[2]	40	**38**	**65**	**65**	**45**	**40**
C[2]	13	**38**	**46**	**62**	**38**	**31**
All subjects	53	**38**	**60**	**64**	**43**	**38**

[1] Detection rates for daily users were significantly greater than for non-daily users (1-5 times/week) regardless of method designating a positive specimen ($p < 0.05$, Fisher's exact test).

[2] AA African American, C Caucasian. Detection rates were not significantly different between these groups ($p > 0.3$, Fisher's exact test).