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Oral fluid cannabinoid concentrations following controlled smoked cannabis in chronic frequent and occasional smokers

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Abstract

Background—Oral fluid (OF) is an alternative biological matrix for monitoring cannabis intake in drug testing, and drugged driving (DUID) programs, but OF cannabinoid test interpretation is challenging. Controlled cannabinoid administration studies provide a scientific database for interpreting cannabinoid OF tests.

Methods—We compared differences in OF cannabinoid concentrations from 19h before to 30h after smoking a 6.8% THC cigarette in chronic frequent and occasional cannabis smokers. OF was collected with the Statsure Saliva Sampler™ OF device. 2D-GC-MS was used to quantify cannabinoids in 357 OF specimens; 65 had inadequate OF volume within 3h after smoking.

Results—All OF specimens were THC-positive for up to 13.5h after smoking, without significant differences between frequent and occasional smokers over 30h. CBD and CBN had short median last detection times (2.5–4h for CBD and 6–8h for CBN) in both groups. THCCOOH was detected in 25 and 212 occasional and frequent smokers' OF samples, respectively. THCCOOH provided longer detection windows than THC in all frequent smokers. As THCCOOH is not present in cannabis smoke, it's presence in OF minimizes the potential for false positive results from passive environmental smoke exposure, and can identify oral THC ingestion, while OF THC cannot. THC≥1μg/L, in addition to CBD≥1μg/L or CBN≥1μg/L suggested recent cannabis intake (≤13.5h), important for DUID cases, whereas THC≥1μg/L or THC≥2μg/L cutoffs had longer detection windows (≥30h), important for workplace testing. THCCOOH windows of detection for chronic, frequent cannabis smokers extended beyond 30 h, while they were shorter (0–24h) for occasional cannabis smokers.

Keywords

Tetrahydrocannabinol; Cannabinoids; 11-nor-9-carboxy-tetrahydrocannabinol; Oral fluid; Statsure Saliva Sampler; Drug testing

Introduction

Cannabis, also known as marijuana or hashish, is the most widely consumed drug in the world, with a prevalence between 2.6 and 5% in 2010 [1]. In the United States in 2011, 18.1 million people smoked cannabis in the last past month [2]. Cannabis also was the most

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common illicit drug found in drivers, according the 2007 National Roadside Survey of Alcohol and Drug Use by Drivers [3–4].

Saliva or oral fluid (OF) is now extensively utilized in many different drug testing programs [5] including pain management [6], workplace [7], and driving under influence of drugs (DUID) testing [8]. The main advantages of OF are the simplicity and noninvasiveness of samples collected under observation making adulteration more difficult [9].

Determining OF cannabinoid pharmacokinetics after controlled cannabinoid administration is important to determine drug detection windows, establish markers that can distinguish cannabis intake from environmental smoke contamination, and identify new markers to improve OF interpretation. However, ideal drug detection windows are different depending on the purpose of monitoring. For workplace drug testing, detection windows should be as long as possible due to widely separated specimen collections, while shorter detection windows identifying recent use may be needed for human performance testing in forensic, road safety or antidoping fields.

Passive environmental contamination from cannabis-laden smoke is another problem to consider when interpreting OF drug testing results. Environmental cannabis smoke exposure produced positive peak OF Tetrahydrocannabinol (THC) concentrations (up to 1.2 μg/L) in 4 non-smokers present in a van with 4 individuals smoking a 10.4% THC cigarette 1.5h after cessation of smoking, when OF was collected outside the van with the Intercept oral fluid device [10]. More recently, Moore *et al.* showed that up to 17μg/L THC and Cannabinol (CBN) were quantified in OF after 3h environmental smoke exposure in Dutch coffee shops, but 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) was not found in cannabis smoke, making this analyte a good marker for differentiating acute passive environmental exposure from cannabis smoking [11].

In order to provide a scientific database for interpreting cannabinoid OF tests, we conducted controlled oral [12–15], submucosal [12] and smoked cannabinoid administration studies [16–17], and cannabinoid excretion studies during sustained abstinence [18]. However, many participants were chronic, frequent smokers and all studies utilized expectorated OF and/or OF collected with the Quantisal™ device.

Smoking topography (i.e., duration and depth of inhalation and exhalation, hold time in the lungs, and time between puffs) is affected by drug use frequency and chronicity, and potential tolerance development that influences THC plasma concentrations, and probably also oral cavity contamination [19–21]. Furthermore, chronic, frequent use of cannabis leads to accumulation of highly lipophilic THC in tissues, producing a gradual prolonged excretion of THC, and of its metabolite THCCOOH into blood [22–24]. Moreover, OF and plasma THCCOOH concentrations are correlated [25]; consequently, differences in OF THCCOOH concentrations in chronic, frequent and occasional cannabis smokers may be observed, as is seen in plasma.

Toennes *et al.* compared THC OF pharmacokinetics over 8h in occasional and chronic cannabis smokers following smoking of 500μg/kg THC [26]. OF was collected with the Intercept DOA Oral Specimen Collection Device. No significant differences were observed between these two groups over this short time frame except for maximum THC concentration (C_{max}).

In the present study, OF was collected with the Saliva Statsure Sampler™ device utilized in the Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) European Union project. We quantified THC, 11-hydroxy-tetrahydrocannabinol (11-OH-THC), THCCOOH, Cannabidiol (CBD) and CBN OF concentrations prior to and up to 30h after

ad-libitum smoking of a single 6.8% cannabis cigarette in chronic, frequent and occasional cannabis smokers. Comparison of OF cannabinoid disposition in these two cannabis smoker populations, analyte detection windows and potential cutoffs for different drug testing programs were evaluated to provide a scientific database to assess individual OF cannabinoid results.

Experimental

Study design

This clinical study, approved by the National Institute on Drug Abuse Institutional Review Board, recruited chronic frequent cannabis smokers (n=14) who smoked at least four times per week over the last year, and occasional smokers (n=10) who smoked less than twice a week. All participants were between 18–45 years old and provided written informed consent. Additional inclusion criteria included: systolic blood pressure ≤140mmHg, diastolic blood pressure ≤90mmHg, heart rate ≤100 beats per minute, an electrocardiogram 3-min rhythm strip without clinically relevant abnormalities, and peripheral veins suitable for venipuncture. Exclusion criteria included history or presence of any clinically significant illness, adverse event associated with cannabis intoxication, donation of more than 450mL blood within 30 days, interest or participation in drug abuse treatment within 60 days, and for women, pregnancy or nursing. The study consisted of a three day, two-night stay on a closed clinical research unit. Baseline measures and biological samples were collected before drug administration. Participants smoked ad-libitum (10min maximum) one 6.8±0.2% (54 mg) THC, 0.25±0.08 CBD, and 0.21±0.02% CBN cannabis cigarette the morning of Day 2 and provided OF up to 30h after smoking.

Biological sample collection and analysis

The Statsure Saliva Sampler™ device (StatSure Diagnostic Systems), utilized for OF collection has an absorptive cellulose pad, a volume adequacy indicator that turns blue upon collection of 1.0mL OF, and a polypropylene tube containing 1mL elution/stabilizing buffer, yielding a 1:1 OF dilution. OF was collected 19 and 1h before and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10.5, 13.5, 21, 24, 26, 28, 30h after smoking. All samples were stored at 4°C and analyzed within 24h of collection. No weight correction was applied and, in the case of low OF volume, specimens were analyzed, and as we discuss, these concentrations were likely underestimated due to a greater dilution with elution buffer.THC, 11-OH-THC, THCCOOH, CBN, and CBD were quantified by two-dimensional gas chromatography mass spectrometry (2D-GCMS) according to a previously published method [27] with minor modifications: calibrators and quality controls were prepared in 0.25mL blank OF and 0.25mL StatSure buffer to account for OF dilution. Also, GC column configuration for neutral cannabinoid analysis was changed to that of our plasma method [28] so that GC instruments could flexibly be utilized for either analysis; instruments were equipped with DB-1MS (Agilent Technologies) as the primary column and ZB-50 (Phenomenex) as the secondary column Limits of quantification (LOQ) were 0.5μg/L for THC, 11-OH-THC, CBD and CBN, and 15ng/L for THCCOOH. Upper LOQ were 50μg/L for THC, 11-OH-THC, CBD and CBN, and 500ng/L for THCCOOH. The modified method's performance was comparable to that of the original method with 1–2.7%RSD intra-assay imprecision (n = 6), 2.2–7.6%RSD inter-assay imprecision (n = 10). OF specimens were diluted with drug free OF-Statsure buffer mixture if analyte concentrations exceeded the upper LOQ.

Data Analysis

Data were processed with Chemstation Data Analysis software (Agilent Technologies, Wilmington, DE, USA) to generate analyte concentrations. Quantification was carried out by linear regression with 1/x weighting. Low, medium and high concentration quality

controls across the dynamic range of the assay were incorporated to ensure analytical quality throughout the run. IBM SPSS Statistics version 18.0 for Windows and Microsoft Excel were employed in statistical evaluations. Group medians were compared with the non-parametric Mann-Whitney U test, p-value <0.05 indicated no significant difference between groups. When comparing median group concentrations at each time point (n=17), Bonferroni correction reduced type 1 errors; consequently, p <0.003 indicated significant difference between daily and occasional smokers. Areas under the curve (AUC) were estimated with Excel by the trapezoid method. For figures, values below the LOQ were considered as one tenth the LOQ.

Results

Fourteen chronic, frequent and 10 occasional cannabis smokers spent the night before smoking on the clinical unit to ensure that participants were not intoxicated at the time of smoking. OF specimens (N=382) were collected prior to and up to 30h after cannabis smoking. Within 3h after smoking, 54 or 56% of specimens collected in this time frame had inadequate volume due to reduced salivation or “dry mouth.” Low OF volume was noted in 22 of 24 specimens at 0.5h, 18 at 1h, 11 at 2h and 3 at 3h after smoking. Overall, 19% (chronic frequent smokers) and 17% (occasional smokers) of all OF specimens had an inadequate volume. There were no significant differences in the percentage of low volume specimens or the length of time low volume specimens were obtained between groups.

Table 1 presents demographics and self-reported cannabis smoking history for 24 participants. Median (range) age of first cannabis smoking, lifetime years of smoking, and body mass index (BMI) were 14 (11–25) years, 10.5 (4.4–17.6) years and 26.1 (19.4–35.4) for the chronic frequent smokers and 16 (13–19) years, 17.7 (7.7–25.3) years and 24.7 (21–47.8) for occasional users. No significant differences were observed between chronic, frequent and occasional smokers for these parameters (p=0.522 for age of first use, and p=0.738 for body mass index) except for the lifetime smoking (p=0.038). A major difference between these groups was, as expected, the median number of joints smoked per week. Chronic, frequent smokers reported a median (range) of 28 joints per week (21–147), while occasional smokers reported 0.75 joints per week (0.06–2.5). Consequently, the number of days since last cannabis smoking also was different among groups, as almost every chronic frequent smoker reported intake the day prior to smoking (day of admission), whereas the last time for occasional smokers varied between 3 to 87 days. Two participants (M and N) were originally classified as occasional users by their self-report, but following analysis of biological specimens, reclassified as chronic, frequent smokers based on our previous findings [16,29].

Figure 1 shows the median time course of THC, THCCOOH, CBD and CBN, while Table 2 describes the percentage of positive OF specimens (≥LOQ) for each of these analytes in the two groups. Data for 11-OH-THC are not included in the figures or tables because 98% of OF specimens were negative. 11-OH-THC was only present in 10 specimens at the method’s LOQ of 0.5μg/L, with concentrations ranging between 0.6–2.6μg/L. At admission, 19h before controlled smoked cannabis, all OF specimens from occasional smokers were negative for all analytes. In chronic frequent smokers, 13 and 14 OF specimens were positive for THC and THCCOOH, with concentrations between 6–396μg/L and 23–407ng/L, respectively. Interestingly, 43 and 64% of OF specimens were positive for CBD (0.6–7.9μg/L) and CBN (0.9–16.1μg/L). One h prior to cannabis smoking, concentrations in frequent smokers’ OF had decreased; no specimens were positive for CBD or CBN, and 11 were still positive for THC (0.9–7μg/L). THCCOOH OF concentrations decreased 35% from 19 to 1h prior to smoking, but 100% remained positive (20–223ng/L) 1h before controlled smoking began.

After smoking, OF THC concentrations increased rapidly in both groups with median C_{max} concentrations 0.5h post smoking (first collection time point) of 517 (189–6508)μg/L for chronic, frequent and 524 (85–1471)μg/L for occasional cannabis smokers, although many of these were low volume collections suggesting underestimated concentrations. Two occasional smokers had THC t_{max} at 1 and 2 h after smoking, corresponding to C_{max} of 561μg/L and 1080μg/L, respectively. This could be explained by low OF volumes in specimens collected 0.5h after smoking for these two participants that would have generated a greater than 1:1 dilution with the buffer. Generally, THC OF concentrations progressively decreased and median concentrations fell from 218 (28.4–2354)μg/L 1h after smoking to 71.1 (7.5–350)μg/L after 2h in chronic, frequent smokers as compared to 93.6 (48.4–561)μg/L to 78.3 (23.4–1080)μg/L within the same interval in occasional smokers. 13.5h after smoking, 100% of 24 OF specimens were still THC positive with median OF concentrations of 2.8 (0.8–18.4) for frequent and 1.8 (0.8–34.5)μg/L for occasional cannabis smokers. Median THC last detection times for frequent and occasional smokers were >30 (13.5->30) and 27 (21->30)h, respectively, documenting no significant differences (p=0.067) up to 30h. There also were no significant differences between median areas under the curve ($AUC_{0\rightarrow30h}$) (Table 3), 550 (176.9–4179) and 556 (139–1674)μg/L.h for frequent and occasional smokers (p=0.539), revealing similar time courses as seen in Figure 1.

CBD and CBN had similar time courses to THC's, but with lower concentrations. CBD and CBN C_{max} also occurred at 0.5h for all participants except for the 2 participants with late THC C_{max}. For these participants, C_{max} occurred, as for THC, at 1 and 2h. After 0.5h, median CBD and CBN OF concentrations were 25 (4.5–254.5) and 36 (7–476)μg/L for frequent cannabis smokers and 11 (1.9–42) and 37 (6.8–138)μg/L for occasional smokers. OF concentrations decreased rapidly yielding last CBD detection times of 4 (1–10.5) and 2.5 (2–6)h, respectively, for frequent and occasional smokers. Detection windows for CBN were longer, with last positive OF tests in frequent and occasional smokers of 8 (2–28) and 6 (2–13.5)h. As for THC, $AUC_{0\rightarrow30h}$ for CBD (p=0.497) and CBN (p=0.771) showed no significant differences between frequent and occasional smokers.

THCCOOH concentrations had the largest differences between the two smoking groups. 97% of all frequent smokers' OF specimens were positive, while only 15% of occasional smokers' specimens were positive. For frequent smokers, median THCCOOH C_{max} was 126 (59.7–430)ng/L with an associated t_{max} of 1 (0.5–10.5)h. Although a small decrease in THCCOOH concentrations was observed over time, 85% of OF specimens remained positive 30h after smoking at a median concentration of 25.5 (<LOQ-197)ng/L. For occasional smokers 5h after cannabis intake, THCCOOH median was 17.6 (<LOQ-38.3)ng/L; however, overall, few specimens were positive and median THCCOOH concentration was 0ng/L at all other time points. Furthermore, specimens were randomly positive and one or more negative specimens were found between two positive specimens.

The Substance Abuse and Mental Health Services Administration (SAMHSA) proposed a 2μg/L THC confirmation cutoff; all participants' OF specimens were positive for 6h. However, at 21h after smoking, only 71% of frequent smokers and 10% of occasional smokers' specimens were positive. At the 1μg/L cutoff utilized by the DRUID program, all participants were positive for 10.5h. At 26h, 7 frequent smokers were positive; only 1 occasional smoker had THC concentrations >1μg/L. In addition to DRUID and SAMHSA cutoffs, THCCOOH≥20ng/L alone or in combination with THC≥1 and THC≥2μg/L were evaluated. Combinations of THC≥1μg/L + CBD ≥1μg/L or CBN ≥1μg/L also were investigated (Figure 2).

With a THCCOOH≥20ng/L cutoff, 64% of frequent smokers' specimens were still positive at 30h. When cutoffs of THCCOOH≥20 ng/L and THC≥1 μg/L were applied, the number of

positives for frequent smokers decreased linearly after 10.5h, with 1 specimen positive at 30h, greatly reducing the window of detection. When in combination with THC≥2μg/L, the number of positive specimens started to decrease at 6h, and 1 specimen remained positive at the last collection (30h). For occasional smokers, 90% of all collected OF were negative with a THCCOOH≥20ng/L cutoff. With a THCCOOH cutoff ≥20ng/L or THC≥2μg/L considered by SAMSHA, 69% of chronic frequent smokers were still positive at 30h after smoking, while last detection time for occasional smokers was 24h with 30% positive. With THC≥1μg/L and CBD≥1μg/L cutoff, windows of detection were short for both groups; 3h after smoking, only 6 participants (5 frequent and 1 occasional smokers) remained positive. Using a THC≥1μg/L and CBN≥1μg/L cutoff, 45% of frequent and 30% of occasional smokers were positive 6h post-smoking.

Discussion

We present the disposition of THC, THCCOOH, CBD and CBN in OF after controlled smoking of a 6.8% THC cigarette in 14 chronic, frequent and 10 occasional cannabis smokers. These also are the first OF data after controlled cannabis smoking for the StatSure Saliva Sampler device. Only one other study compared chronic and occasional cannabis smokers, but only evaluated THC OF disposition for 8h [26], and collected OF with the Intercept DOA Oral Specimen Collection Device.

Before smoking, there was a substantial difference in OF results between the chronic frequent and the occasional smokers. On admission, all OF specimens from occasional smokers were negative for all analytes, while most OF specimens from chronic frequent smokers were positive, confirming recent cannabis intake based on our previous controlled cannabis administration [16–17] and sustained abstinence studies [18]. During the 18h overnight stay, CBD and CBN concentrations decreased until none were positive 1 h prior to smoking. However, OF specimens remained positive for THC and THCCOOH due to residual excretion of previously self-administered smoked cannabis.

Immediately after smoking, high THC, CBD and CBN concentrations were observed reflecting contamination of the oral mucosa by cannabis smoke. Cannabinoid OF concentrations varied with cannabis plant cannabinoid composition, smoking topography, side stream loss, drug left in the cigarette butt and THC pyrolysis during the burning process [19,30]. In this study, *ad-libitum* smoking introduced more inter-subject variability, but reflected more naturalistic results than paced smoking protocols, and was especially important for evaluating differences between frequent and occasional cannabis smokers.

Our THC median C_{max} and t_{max} in chronic frequent smokers (Table 2) were respectively lower and later than other studies involving frequent smokers due to the later time of first specimen collection. When we collected OF was collected by expectoration or with the Quantisal™ device after smoking a 6.8% THC cigarette, Cmax were obtained in both cases at 0.25h and were respectively 265–22,370μg/L and 68–10,284μg/L [16–17]. When collected with the Intercept™ device, THC C_{max} was 387–71,747μg/L 0.08min after smoking a cannabis cigarette containing 500μg/kg or about 35mg THC [26]. These differences are easily explained by times of first OF collection, 0.25 and 0.08h in the cited studies, and 0.5h in our study. We chose to delay first collection due to the high number of inadequate volume OF specimens close to the time of smoking noted in our earlier studies [16–17]. Reduced salivation or dry mouth frequently occurs immediately after cannabis smoking. OF THC concentration drops rapidly after smoking, accounting for C_{max} differences across studies. However, our concentrations at 0.5h are similar to previous reports: Lee *et al.* utilized the Quantisal™ device and reported concentrations of 40–6,362μg/L 0.5h after smoking a 6.8% THC cigarette, and after the same dose, expectorated

OF concentrations were 311–1,524μg/L 0.5h post-smoking [16–17]. For occasional smokers, only one study reported OF THC concentrations between 397–6,438μg/L only 0.08h (5 min) after smoking a cannabis cigarette containing an equivalent of 500μg/kg or about 35 mg THC [26]. We observed THC concentrations between 84.5–1,471.3μg/L at the first sampling (0.5h). No significant differences (p=0.284) were observed for C_{max} in our study across groups. At our t_{max} (0.5h), Toennes *et al* showed no significant difference in THC concentrations in frequent compared to occasional cannabis smokers due to a more rapid decrease in THC concentrations in frequent smokers from 5 to 15min (19). However, in the Toennes study the Intercept device, utilized for OF collection, has no volume adequacy indicator. It is possible that low volume OF collections could have influenced the rate of decline in THC concentrations. Thus, the proposed more rapid decrease in THC concentrations over time in frequent smokers should be verified in specimens of known OF volume. In our study, THC concentrations decreased to medians of 12 (2.5–27.4) and 8.3 (2.7–27.8)μg/L at 6h for frequent and occasional smokers, respectively. This is in accord with other studies reporting concentrations between 2.1–44μg/L at a similar time [31,16]. Between 24 and 30h after smoking, more than 73% of frequent smokers' OF specimens were still THC positive; these windows of detection match those reported previously [32,16,33]. Toennes *et al* [26] showed no significant differences in AUC for 8h (last collection time) after smoking between 12 frequent and 12 occasional smokers. In our study, we extended monitoring to 30h with no significant differences in AUC (p = 0.539) between chronic and occasional smokers (Table 3).

For our chronic frequent cannabis smokers, CBD and CBN C_{max} concentrations occurred 0.5h after smoking (Table 3), in the range previously described by Lee *et al.* (2.6–588μg/L for CBD and 4.8–1,558μg/L for CBN) and Milman *et al.* (8.8–1,000μg/L for CBD and <LOQ to 1,954μg/L for CBN) [16–17]. Observed differences in CBD results could not be due to differences in cigarette CBD content because both the prior and current study employed cannabis cigarettes with identical CBD content. Therefore, observed differences could more likely be explained by inter-individual variations, smoking topographies, OF collection devices (Quantisal in Lee's study and expectorated OF in Milman's), OF volume collected and/or collection times.

These are the first OF data for CBD and CBN in occasional smokers after controlled smoked cannabinoid administration; thus, no comparison with previous reports is possible. We found no statistically significant differences in CBD and CBN C_{max} (p = 0.313 and 0.376 respectively) and AUC (p = 0.497 and 0.771) between occasional and frequent smokers. In addition, CBD concentrations in the cannabis cigarette were approximately 3.5% of THC's (or 0.25% of cigarette total weight), similar to the relative amount present in the cannabis cigarette. This reflects a similar oral mucosa contamination for CBD and THC. In the 6.8% THC cigarette, CBN was 3% of THC's content (or 0.21% total weight); however in OF specimens, CBN concentration is approximately 8% of THC's. CBN is a degradation product of THC; therefore, its concentration may be influenced by the conversion of THC to CBN during smoking.

Since THCCOOH is not present in cannabis smoke, OF concentrations must reflect THC metabolism in the liver or oral mucosa. CYP2C9 enzymes, important for formation of 11-OH-THC and THCCOOH, were identified in the liver [34–35], and also in human buccal, and tongue cells [36–37]. For frequent smokers, THCCOOH was present in 97% of specimens with a last detection time of ≥30h. THCCOOH peak concentrations occurred between 0.5 and 10.5h after smoking, with a 2h median (Table 3), and were similar to those reported in other studies, <1,118ng/L after 37 oral THC doses or <764ng/L after one 6.8% THC cigarette [16,14]. In contrast, many OF specimens from occasional smokers were THCCOOH-negative. THCCOOH glucuronide also was reported in OF [38–39]; our

analytical procedure did not include a hydrolysis step, and thus, results may have been higher following OF hydrolysis. Additional research is required to evaluate THCCOOH-glucuronide concentrations in frequent and occasional cannabis smokers after controlled smoked cannabis administration.

THC and THCCOOH last detection times are difficult to compare across devices in our studies despite smoking of an identical potency cannabis cigarette, because participants were different, monitoring windows were 22h for Quantisal and expectoration and 30 h for StatSure, and cutoffs varied. Also, windows of detection in chronic frequent smokers generally exceeded the duration of monitoring for THCCOOH in all studies. However, some comparisons are possible. First, the StatSure Saliva Sampler™ produced, within 3h after smoking, more low OF volume specimens due to dry mouth than the Quantisal™ device (56% vs. 43%), at identical time points of collection [16]. The StatSure Saliva Sampler™ (1:1 v/v) dilutes the sample less than the Quantisal™ device (1:3 v/v), increasing sensitivity, and perhaps increasing detection rates and last detection times. At the same cutoffs, THC (0.5μg/L cutoff) and THCCOOH (15ng/L cutoff) last detection rates (86% and 93%, respectively) were higher in OF collected with the StatSure Saliva Sampler™ at 24h, compared to 67% and 50% at 22 h, respectively, when OF was collected with the Quantisal™ device. CBD (0.5μg/L cutoff) and CBN (1μg/L cutoff) last detection times were similar between studies with 30% and 36% positive specimens at 6h and none positive at 22h. However, the low StatSure OF dilution is a disadvantage as far as total OF/buffer volume available for confirming presumptive positive screening tests, or for multiple chromatographic assays.

Compared to expectorated OF specimens, the StatSure Saliva Sampler™ device had more low volume specimens [17]. Comparison of THC and THCCOOH last detection times is difficult for the same reasons as described earlier when the StatSure Saliva Sampler™ device was compared to the Quantisal™ device. Overall, there were more positive CBD and CBN specimens when OF was collected by expectoration, due to lack of dilution enhancing sensitivity. However, we recently reported that cannabinoid stability in expectorated OF was poorer than when collected with a stabilizing buffer [40]. In the study by Milman *et al.*, OF specimens were analyzed within 24h to preclude degradation, thus, these results may not be predictive of other studies with delayed analyses.

Our laboratory recently evaluated different OF cannabinoid cutoff concentrations in frequent smokers during 4–33 days of abstinence and following smoking of a single 6.8% THC cigarette [18,16]. These cutoffs were based on CBD, CBN or THCCOOH concentrations in combination with THC, or on THCCOOH and THC concentrations alone. For frequent smokers, a combination of THC≥1–2 μg/L and CBD or CBN≥1μg/L provided detection windows shorter than 6h. These short detection windows also were confirmed for chronic frequent and occasional smokers in the current study (Figure 2). Detection windows were dependent on CBD and CBN concentrations, not THC concentrations; thus, THC thresholds of ≥2 or ≥1μg/L in addition to CBD or CBN≥1 μg/L did not affect last detection times. Using CBN instead of CBD provided longer windows of detection with 45% of frequent and 30% of occasional smokers positive 6h post-smoking. These results showed the importance of measuring CBN and CBD since CBN or CBD cutoff may be appropriate when proof of recent cannabis consumption is needed, for example in DUID or anti-doping cases when human performance may be impaired by cannabis intake. However, the absence of CBD and CBN does not preclude recent cannabis smoking, as many OF specimens were negative in frequent and occasional cannabis smokers by 2h after smoking. Moreover, CBD and CBN content in cannabis cigarettes are variable; thus, windows of detection may vary with cannabinoid cigarette content.

A positive OF CBD and CBN, both present in cannabis smoke, could be due to passive environmental smoke exposure. In the only passive cannabis smoke exposure study measuring CBD and CBN, 3 of 10 subjects exposed for 3h had up to 2μg/L CBN in their OF [11]. CBD was not detected at an LOQ of 1μg/L, possibly due to low CBD content in cannabis cigarettes. THC cutoff concentrations alone (SAMHSA's 2μg/L or DRUID's 1μg/L) provided longer detection windows: up to 26 and 30h for frequent smokers and up to 24 and 26h for occasional smokers, but cannot exclude acute passive environmental smoke contamination. In addition, THC as the only monitored analyte, would not detect synthetic oral THC intake, and has a shorter detection window in chronic frequent smokers than THCCOOH [14].

There also are advantages and disadvantages to monitoring only THCCOOH in OF. For advantages, Moore *et al.* reported the absence of THCCOOH after acute passive environmental exposure when THC and CBN were present [11], and we reported that OF THCCOOH followed the time course of plasma THCCOOH after controlled oral THC administration [13–15].THCCOOH≥20ng/L provided a higher detection rate (number of specimens positive divided by the total number of specimens collected) in chronic frequent cannabis smokers compared to THC at 1 or 2 μg/L after controlled oral THC administration [14,13,12], after controlled smoked THC [16–17,41,10], during sustained abstinence [18] and in random cannabis smokers previously positive for THC [42–43]. However, fewer occasional smokers' OF specimens were THCCOOH-positive. Consequently, a cutoff based on THCCOOH in OF documents cannabis intake, but its absence in occasional cannabis smokers' OF does not exclude cannabis smoking. Quantifying THCCOOH at low ng/L concentration range is challenging and requires development of highly sensitive assays. However, multiple assays meeting this sensitivity requirement are available that employ different analytical systems: two-dimensional GC-MS [27,44], GC-MS/MS [42], LC-MS/MS [45–47] and LC-HRMS [48–49]. These findings confirm the advantages of monitoring multiple cannabinoid markers to improve interpretation of cannabinoid OF results.

Conclusion

These are the first OF cannabinoid data after controlled smoked cannabis administration with OF collection by the Statsure Saliva Sampler device. Moreover, THC, THCCOOH, 11-OH-THC, CBD and CBN OF disposition is compared for the first time after controlled smoked cannabis administration in two different populations, chronic frequent and occasional cannabis smokers. In both groups, maximum THC, CBD and CBN concentrations occurred just after smoking. No statistically significant differences in the overall cannabinoid time course were observed between frequent and occasional smokers, except for THCCOOH. THCCOOH identified fewer occasional cannabis smokers than THC, but its presence rules out acute passive environmental exposure. Additional research is needed to determine if hydrolysis of THCCOOH-glucuronide would improve detection rates and windows of detection in occasional cannabis smokers. Advantages and disadvantages for setting cannabinoid cutoff concentrations in OF and selecting different OF markers of cannabinoid exposure were described, each providing unique information on an individual's drug use history. Interpretation of cannabinoid OF results is complex due to different routes of drug exposure, potential oral mucosal contamination, and different frequencies and chronicities of exposure.

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Abbreviations

OF	Oral fluid
SAMHSA	Substance Abuse and Mental Health Services Administration
DUID	Driving Under Influence of Drugs
DRUID	Driving Under the Influence of Drugs, Alcohol and Medicines
LOQ	Limit of Quantification

References

1. United Nations Office on Drugs and Crime (UNODC). World Drug Report 2011. Vienna: 2011.
2. Substance Abuse and Mental Health Services Administration (SAMHSA). U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES SAaMHSA, Center for Behavioral Health Statistics and Quality. Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings. Substance Abuse and Mental Health Services Administration; Rockville, MD: 2011.
3. Substance Abuse and Mental Health Services Administration. NSDUH Series H-34. Department of Health and Human Services (DHHS); Rockville, MD: 2008. Results from the 2007 National Survey on Drug Use and Health: National Findings.
4. Lacey JH, Kelley-Baker T, Romano E, Brainard K, Ramirez A. Results of the 2012 California Roadside Survey of Nighttime Weekend Drivers'. Alcohol and Drug Use. 2012
5. Huestis MA, Verstraete A, Kwong TC, Morland J, Vincent MJ, De La Torre R. Oral fluid testing: Promises and pitfalls. Clinical Chemistry. 2011; 57 (6):805–810. [PubMed: 21350039]
6. Heltsley R, Depriest A, Black DL, Crouch DJ, Robert T, Marshall L, Meadors VM, Caplan YH, Cone EJ. Oral fluid drug testing of chronic pain patients. II. Comparison of paired oral fluid and urine specimens. J Anal Toxicol. 2012; 36(2):75–80. bkr019 [pii]. 10.1093/jat/bkr019 [PubMed: 22337775]
7. Tsanaclis LM, Wicks JFC, Chasin AAM. Workplace drug testing, different matrices different objectives. Drug Testing and Analysis. 2012; 4 (2):83–88.10.1002/dta.399 [PubMed: 22362574]
8. Verstraete AG. Oral fluid testing for driving under the influence of drugs: history, recent progress and remaining challenges. Forensic Science International. 2005; 150 (2–3):143–150. [PubMed: 15944054]
9. Bosker WM, Huestis MA. Oral Fluid Testing for Drugs of Abuse. Clin Chem. 2009; 55 (11):1910–1931. [PubMed: 19745062]
10. Niedbala RS, Kardos KW, Fritch DF, Kunsman KP, Blum KA, Newland GA, Waga J, Kurtz L, Bronsgeest M, Cone EJ. Passive cannabis smoke exposure and oral fluid testing. II. Two studies of extreme cannabis smoke exposure in a motor vehicle. Journal of Analytical Toxicology. 2005; 29 (7):607–615. [PubMed: 16419389]
11. Moore C, Coulter C, Uges D, Tuyay J, van der Linde S, van Leeuwen A, Garnier M, Orbita J Jr. Cannabinoids in oral fluid following passive exposure to marijuana smoke. Forensic Science International. 2011; 212 (1–3):227–230. [PubMed: 21763088]
12. Lee D, Karschner EL, Milman G, Barnes AJ, Goodwin RS, Huestis MA. Can oral fluid cannabinoid testing monitor medication compliance and/or cannabis smoking during oral THC and oromucosal Sativex administration? Drug Alcohol Depend. 2012 S0376–8716(12)00411–5 [pii]. 10.1016/j.drugalcdep.2012.10.011
13. Milman G, Barnes A, Schwope D, Schwilke E, Goodwin R, Kelly D, Gorelick D, Huestis M. Cannabinoids and metabolites in expectorated oral fluid after 8 days of controlled around-the-clock oral THC administration. Analytical and Bioanalytical Chemistry. 2011; 401 (2):599–607.10.1007/s00216-011-5066-4 [PubMed: 21637933]
14. Milman G, Barnes AJ, Schwope DM, Schwilke EW, Darwin WD, Goodwin RS, Kelly DL, Gorelick DA, Huestis MA. Disposition of Cannabinoids in Oral Fluid after Controlled Around-the-Clock Oral THC Administration. Clin Chem. 2010; 56 (8):1261–1269.10.1373/clinchem.2009.141853 [PubMed: 20530732]

15. Milman G, Schwope DM, Schwilke EW, Darwin WD, Kelly DL, Goodwin RS, Gorelick DA, Huestis MA. Oral Fluid and Plasma Cannabinoid Ratios after Around-the-Clock Controlled Oral {Delta}9-Tetrahydrocannabinol Administration. Clin Chem. 2011; 57 (11):1597–1606.10.1373/clinchem.2011.169490 [PubMed: 21875944]
16. Lee D, Schwope DM, Milman G, Barnes AJ, Gorelick DA, Huestis MA. Cannabinoid Disposition in Oral Fluid after Controlled Smoked Cannabis. Clinical Chemistry. 2012; 58(4)10.1373/clinchem.2011.177881
17. Milman G, Schwope DM, Gorelick DA, Huestis MA. Cannabinoids and metabolites in expectorated oral fluid following controlled smoked cannabis. Clinica Chimica Acta. 2012; 413 (7–8):765–770.
18. Lee D, Milman G, Barnes AJ, Goodwin RS, Hirvonen J, Huestis MA. Oral Fluid Cannabinoids in Chronic, Daily Cannabis Smokers During Sustained, Monitored Abstinence. Clin Chem:clinchem. 2011; 2011:164822.10.1373/clinchem.2011.164822
19. Perez-Reyes, M. Marijuana smoking: factors that influence the bioavailability of tetrahydrocannabinol. In: Chiang, CN.; Hawks, RL., editors. Research Findings on Smoking of Abused Substances. Rockville: 1990. p. 42-62.NIDA Research Monograph #99
20. McClure E, Stitzer M, Vandrey R. Characterizing smoking topography of cannabis in heavy users. Psychopharmacology. 2012; 220 (2):309–318.10.1007/s00213-011-2480-4 [PubMed: 21922170]
21. Azorlosa JL, Greenwald MK, Stitzer ML. Marijuana smoking: Effects of varying puff volume and breathhold duration. Journal of Pharmacology and Experimental Therapeutics. 1995; 272:560–569. [PubMed: 7853169]
22. Toennes SW, Ramaekers JG, Theunissen EL, Moeller MR, Kauert GF. Comparison of Cannabinoid Pharmacokinetic Properties in Occasional and Heavy Users Smoking a Marijuana or Placebo Joint. Journal of Analytical Toxicology. 2008; 32:470–477. [PubMed: 18713514]
23. Bergamaschi MM, Karschner EL, Goodwin RS, Scheidweiler KB, Hirvonen J, Queiroz RH, Huestis MA. Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. Clin Chem. 2013; 59(3):519–526. clinchem.2012.195503 [pii]. 10.1373/clinchem.2012.195503 [PubMed: 23449702]
24. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. Journal of Analytical Toxicology. 1992; 16:276–282. [PubMed: 1338215]
25. Lee D, Vandrey R, Milman G, Bergamaschi M, Mendu DR, Murray JA, Barnes AJ, Huestis MA. Oral fluid/plasma cannabinoid ratios following controlled oral THC and smoked cannabis administration. Anal Bioanal Chem. 201310.1007/s00216-013-7159-8
26. Toennes SW, Ramaekers JG, Theunissen EL, Moeller MR, Kauert GF. Pharmacokinetic Properties of 9-Tetrahydrocannabinol in Oral Fluid of Occasional and Chronic Users. Journal of Analytical Toxicology. 2010; 34 (4):216–221. [PubMed: 20465868]
27. Milman G, Barnes AJ, Lowe RH, Huestis MA. Simultaneous quantification of cannabinoids and metabolites in oral fluid by two-dimensional gas chromatography mass spectrometry. Journal of Chromatography A. 2010; 1217 (9):1513–1521.10.1016/j.chroma.2009.12.053 [PubMed: 20083251]
28. Lowe RH, Karschner EL, Schwilke EW, Barnes AJ, Huestis MA. Simultaneous quantification of delta9-tetrahydrocannabinol, 11-hydroxy-delta9-tetrahydrocannabinol, and 11-nor-delta9-tetrahydrocannabinol-9-carboxylic acid in human plasma using two-dimensional gas chromatography, cryofocusing, and electron impact-mass spectrometry. J Chromatogr A. 2007; 1163:318–327. [PubMed: 17640656]
29. Schwope DM, Karschner EL, Gorelick DA, Huestis MA. Identification of Recent Cannabis Use: Whole-Blood and Plasma Free and Glucuronidated Cannabinoid Pharmacokinetics following Controlled Smoked Cannabis Administration. Clin Chem. 2011; 57 (10):1406–1414.10.1373/clinchem.2011.171777 [PubMed: 21836075]
30. Davis, KH.; McDaniel, IA.; Cadwell, lW; Moody, PL. Some smoking characteristics of marijuana cigarettes. In: Agurell, S.; Dewey, WL.; Willette, RE., editors. The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects. Academic Press; Orlando: 1984. p. 97-109.

31. Kauert GF, Ramaekers JG, Schneider E, Moeller MR, Toennes SW. Pharmacokinetic properties of delta9-tetrahydrocannabinol in serum and oral fluid. Journal of Analytical Toxicology. 2007; 31 (5):288–293. [PubMed: 17579974]
32. Niedbala RS, Kardos KW, Fritch DF, Kardos S, Fries T, Waga J, Robb J, Cone EJ. Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. Journal of Analytical Toxicology. 2001; 25 (5):289–303. [PubMed: 11499881]
33. Huestis MA, Cone EJ. Relationship of Delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. Journal of Analytical Toxicology. 2004; 28 (6):394–399. [PubMed: 15516285]
34. Maurer HH, Sauer C, Theobald DS. Toxicokinetics of drugs of abuse: current knowledge of the isoenzymes involved in the human metabolism of tetrahydrocannabinol, cocaine, heroin, morphine, and codeine. Ther Drug Monit. 2006; 28(3):447–453. 00007691-200606000-00027 [pii]. 10.1097/01.ftd.0000211812.27558.6e [PubMed: 16778732]
35. Watanabe K, Matsunaga T, Yamamoto I, Funae Y, Yoshimura H. Involvement of CYP2C in the metabolism of cannabinoids by human hepatic microsomes from an old woman. Biological & pharmaceutical bulletin. 1995; 18 (8):1138–1141. [PubMed: 8535411]
36. Vondracek M, Xi Z, Larsson P, Baker V, Mace K, Pfeifer A, Tjälve H, Donato MT, Gomez-Lechon MJ, Grafström RC. Cytochrome P450 expression and related metabolism in human buccal mucosa. Carcinogenesis. 2001; 22 (3):481–488.10.1093/carcin/22.3.481 [PubMed: 11238190]
37. Yang S-P, Raner GM. Cytochrome P450 expression and activities in human tongue cells and their modulation by green tea extract. Toxicology and Applied Pharmacology. 2005; 202 (2):140–150. [PubMed: 15629189]
38. Moore C, Rana S, Coulter C, Day D, Vincent M, Soares J. Detection of conjugated 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid. Journal of Analytical Toxicology. 2007; 31 (4):187–194. [PubMed: 17555641]
39. Fabritius M, Staub C, Mangin P, Giroud C. Analysis of cannabinoids in oral fluid by liquid chromatography–tandem mass spectrometry. Forensic Toxicology. 2013; 31 (1):151–163.10.1007/s11419-012-0168-z
40. Lee D, Milman G, Schwope DM, Barnes AJ, Gorelick DA, Huestis MA. Cannabinoid Stability in Authentic Oral Fluid after Controlled Cannabis Smoking. Clinical Chemistry. 2012; 58 (7):1101–1109.10.1373/clinchem.2012.184929 [PubMed: 22532594]
41. Niedbala R, Kardos K, Fritch D, Kardos S, Fries T, Waga J, Robb J, Cone E. Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. J Anal Toxicol. 2001; 25:289– 303. [PubMed: 11499881]
42. Day D, Kuntz DJ, Feldman M, Presley L. Detection of THCA in oral fluid by GC-MS-MS. Journal of Analytical Toxicology. 2006; 30 (9):645–650. [PubMed: 17137523]
43. Moore C, Ross W, Coulter C, Adams L, Rana S, Vincent M, Soares J. Detection of the marijuana metabolite 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid specimens and its contribution to positive results in screening assays. Journal of Analytical Toxicology. 2006; 30 (7): 413–418. [PubMed: 16959132]
44. Moore C, Coulter C, Rana S, Vincent M, Soares J. Analytical procedure for the determination of the marijuana metabolite 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid specimens. Journal of Analytical Toxicology. 2006; 30 (7):409–412. [PubMed: 16959131]
45. Scheidweiler K, Himes S, Chen X, Liu H-F, Huestis M. 11-Nor-9-carboxy- 9-tetrahydrocannabinol quantification in human oral fluid by liquid chromatography–tandem mass spectrometry. Analytical and Bioanalytical Chemistry. 2013:1–9.10.1007/s00216-013-7027-6 [PubMed: 23180075]
46. Coulter C, Garnier M, Moore C. Analysis of tetrahydrocannabinol and its metabolite, 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid, in oral fluid using liquid chromatography with tandem mass spectrometry. J Anal Toxicol. 2012; 36(6):413–417. bks039 [pii]. 10.1093/jat/bks039 [PubMed: 22532488]
47. Lee PD, Chang YJ, Lin KL, Chang YZ. Simultaneous determination of Delta9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta9-tetrahydrocannabinol in oral fluid using

isotope dilution liquid chromatography tandem mass spectrometry. Anal Bioanal Chem. 2012; 402(2):851–859.10.1007/s00216-011-5439-8 [PubMed: 22120041]

48. Concheiro M, Lee D, Lendoiro E, Huestis MA. Simultaneous quantification of Delta(9)-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography-high resolution mass spectrometry. J Chromatogr A. 2013; 1297:123–130. S0021-9673(13)00699-7 [pii]. 10.1016/j.chroma.2013.04.071 [PubMed: 23726246]
49. He X, Kozak M, Nimkar S. Ultra-sensitive measurements of 11-Nor-delta(9)-tetrahydrocannabinol-9-carboxylic acid in oral fluid by microflow liquid chromatography-tandem mass spectrometry using a benchtop quadrupole/orbitrap mass spectrometer. Anal Chem. 2012; 84(18):7643–7647.10.1021/ac3019476 [PubMed: 22900543]

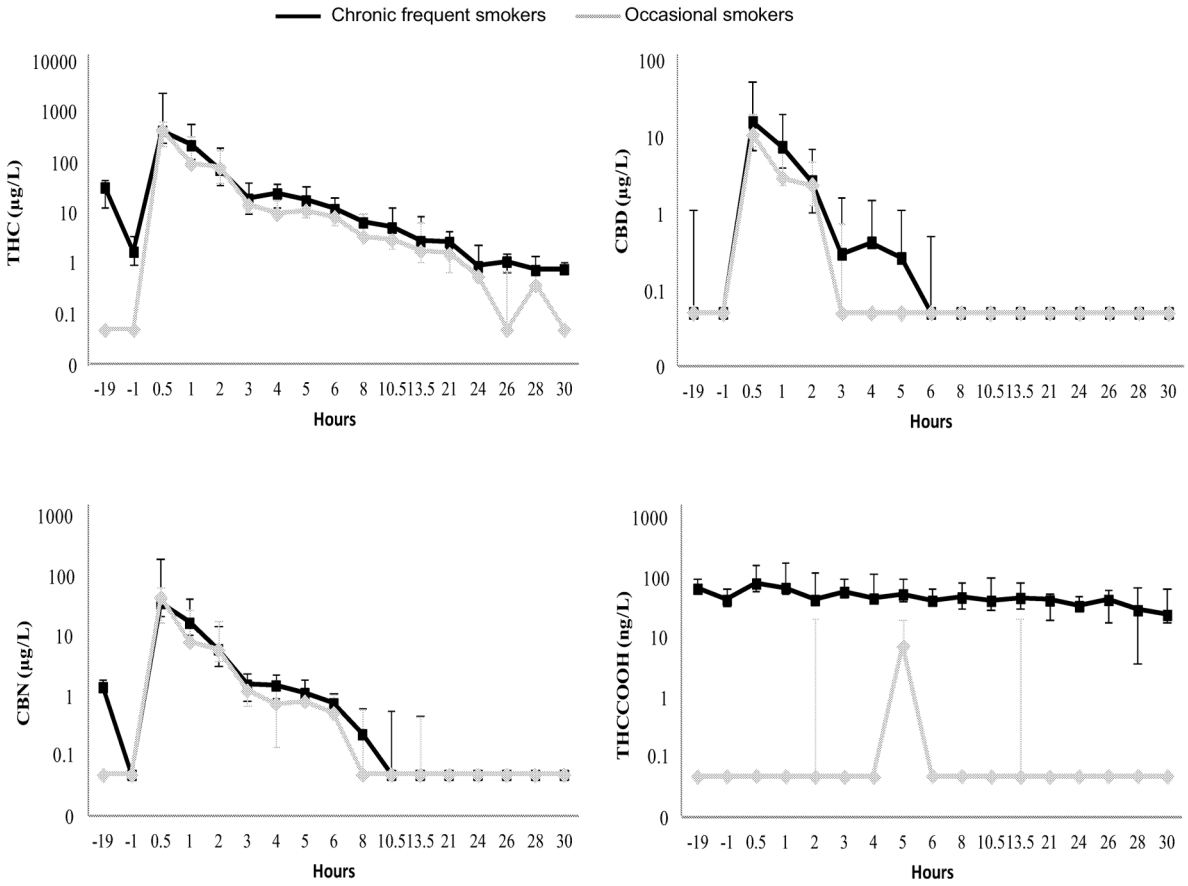

Fig 1.

Median and interquartile ranges for tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN) and 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) oral fluid concentrations for 30h following smoking of one 6.8% THC cigarette in chronic frequent and occasional cannabis smokers. Error bars indicate interquartile ranges

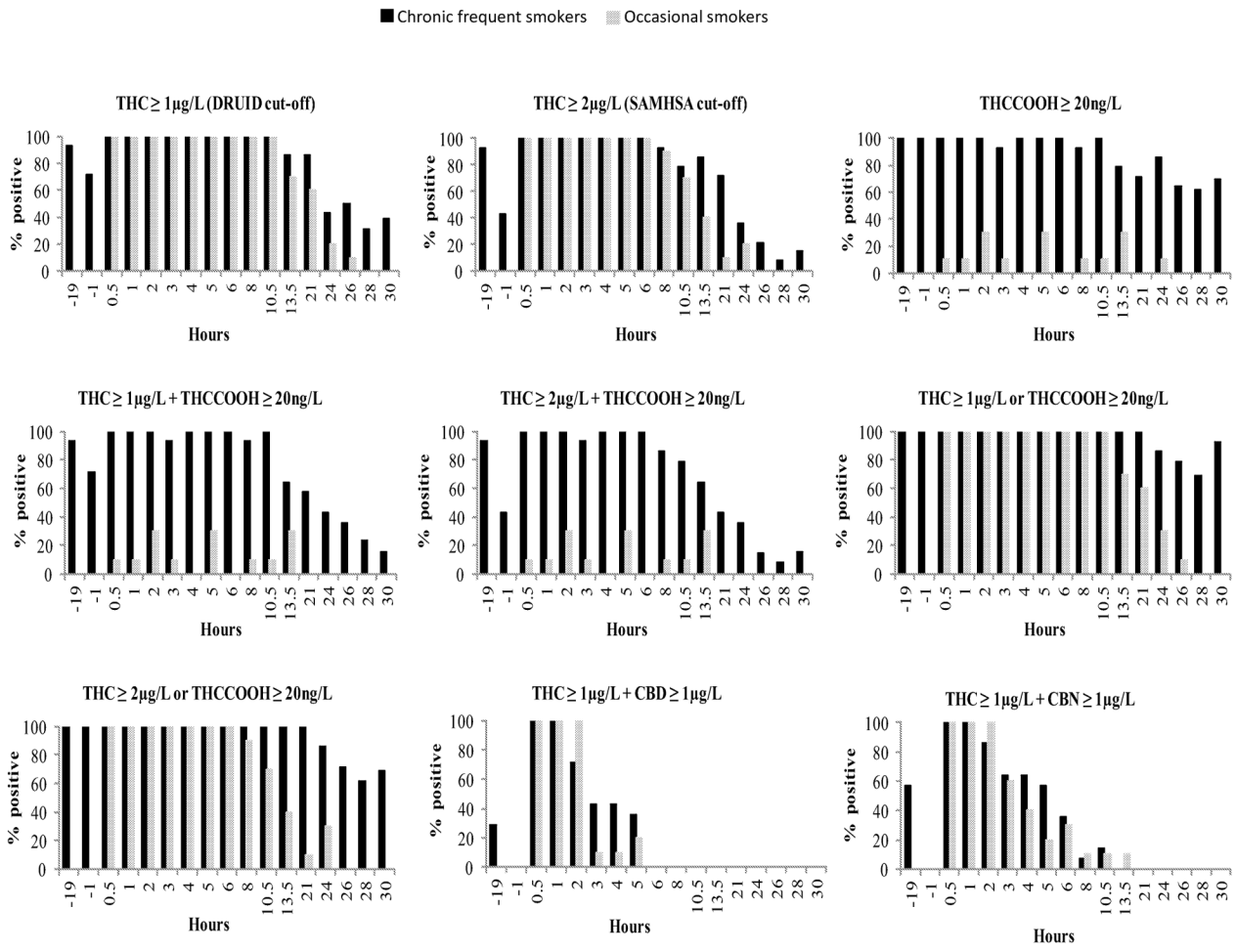

Fig 2.

Percentages of positive specimens at different cutoffs for chronic frequent and occasional cannabis smokers. Cutoffs evaluated were: tetrahydrocannabinol (THC)≥1µg/L (DRUID), THC≥2µg/L (SAMSHA), 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) ≥20 ng/L, THC ≥1µg/L + THCCOOH ≥20 ng/L, THC ≥2µg/L + THCCOOH ≥20 ng/L, THC ≥1µg/L or THCCOOH ≥20 ng/L, THC ≥2µg/L or THCCOOH ≥20 ng/L, THC ≥1µg/L + cannabidiol (CBD) ≥1µg/L and THC ≥1µg/L + cannabinol (CBN) ≥1µg/L

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Table 1

Demographics and self-reported cannabis smoking histories of 24 participants.

Group	Participant	Race [1]	Gender[2]	BodyMass Index	Age	Age 1st use	Lifetime years smoked	Time between last use and dosing (days)	Average joint/week
Chronic frequent smokers	A	AA	M	27.6	29.6	12	17.6	1	28
	B	AA	M	22.6	19.4	15	4.4	1	35
	C	AA	M	31.4	22.6	14	8.6	1	21
	D	W	M	23	25.5	13	12.5	1	140
	E	AA	F	32.4	19.9	11	8.9	1	24.5
	F	AA	M	27.4	24.2	13	11.2	2	10.5
	G	W	F	24.8	22.9	16	6.9	2	42
	H	AA	M	23	37.3	25	12.3	1	21
	I	AA	F	35.4	27.6	18	9.6	1	28
	J	AA	F	20.4	26.9	14	12.9	1	147
	K	AA	M	24.3	23.4	19	4.4	1	48
	L	AA	M	28.1	28.7	14	14.7	1	48
	M	AA	M	19.4	28	14	14	4 [3]	0.5 [3]
	N	AA	M	30.7	23.8	14	9.8	13 [3]	1 [3]
		Median		**26.1**	**24.8** [*]	**14**	**10.5**	**1**	**31.5** [*]
		Min		**19.4**	**19.9**	**11**	**4.4**	**1**	**21**
		Max		**35.4**	**37.3**	**25**	**17.6**	**2**	**147**
Occasional smokers	O	W	M	29.4	25.6	16	9.6	17	0.5
	P	W	M	23.7	25.4	13	12.4	32	0.5
	Q	W	M	24.1	23.7	16	7.7	11	1.75
	R	AA	M	21	38.2	19	19.2	3	0.5
	S	Mixed	M	22	41.3	16	25.3	8	2.5
	T	U	F	31.7	34.9	13	21.9	10	0.5
	U	AA	F	47.8	36.5	18	18.5	3	1
	V	Mixed	M	25.2	22.5	13	9.5	87	1.5
	W	W	F	26.6	34.2	14	20.2	4	0.06
	X	AA	M	21.8	31.7	16	15.7	19	2

Group	Participant	Race [1]	Gender[2]	BodyMass Index	Age	Age 1st use	Lifetime years smoked	Time between last use and dosing (days)	Average joint/week
		Median		**24.7**	**33.0** *	**16**	**17.7**	**10.5** *	**0.8** *
		Min		**21**	**22.5**	**13**	**7.7**	**3**	**0.1**
		Max		**47.8**	**41.3**	**19**	**25.3**	**87**	**2.5**

[1] AA = African American, W = White, U = Unknown

[2] M = male, F= Female

[3] Self-reported data not consistent with biological specimen concentrations. Data excluded from median calculation

* Significant difference ($p<0.05$) observed between chronic frequent and occasional smokers

Table 2

Median percent positive (>Limit of Quantification (LOQ)) specimens and last detection times in chronic frequent and occasional smokers for Tetrahydrocannabinol (THC), Cannabidiol (CBD), Cannabinol (CBN) and 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH). LOQ for THC, CBD and CBN was 0.5μg/L and 15ng/L for THCCOOH.

Time after smoking (h)	THC		CBD		CBN		THCCOOH	
	Daily	Occasional	Daily	Occasional	Daily	Occasional	Daily	Occasional
−19	93	0	43	0	64	0	100	0
−1	79	0	0	0	0	0	100	0
0.5	100	100	100	100	100	100	100	20
1	100	100	100	100	100	100	100	10
2	100	100	79	100	100	100	100	40
3	100	100	50	40	93	80	100	10
4	100	100	50	20	86	70	100	20
5	100	100	50	20	86	80	100	50
6	100	100	29	20	64	60	100	20
8	100	100	7	0	50	40	100	20
10.5	100	100	14	0	29	10	100	10
13.5	100	100	0	0	29	30	100	40
21	93	90	0	0	0	0	93	0
24	86	60	0	0	0	0	93	10
26	79	40	0	0	0	0	100	0
28	64	50	0	0	7	0	71	0
30	64	20	0	0	0	0	79	0
Last detection time (median (range))	>30 (13.5 – >30)	27 (21 – >30)	4 (1 – 10.5)	2.5 (2 – 6)	8 (2 – 28)	6 (2 – 13.5)	>30 (26 – >30)	5 (0 – 24)

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Table 3

C_{max} (maximum concentration observed), t_{max} (time of C_{max}) and $AUC_{0\rightarrow30h}$ (area under the curve from smoking to 30h) of tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN) and 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) and p-value associated to the Mann-Whitney median comparison test between chronic frequent and occasional cannabis smokers.

Analyte	Pharmacokinetic parameters	Chronic frequent smokers median (range)	Occasional smokers median (range)	Mann-Whitney U test p-value [1]
THC	C_{max} (μg/L)	517 (189 – 6508)	533 (84.5 – 1471)	0.284
	t_{max} (h)	0.5 (0.5– 0.5)	0.5 (0.5 – 2)	0.446
	$AUC_{0\rightarrow30h}$ (μg/L.h)	550 (177 – 4179)	556 (139 – 1674)	0.539
CBD	C_{max} (μg/L)	21 (4.5–255)	14.6 (1.9 – 41.1)	0.313
	t_{max} (h)	0.5 (0.5–0.5)	0.5 (0.5 – 2)	0.446
	$AUC_{0\rightarrow30h}$ (μg/L.h)	16.4 (2.1 – 165)	12.2 (2.6 – 40.2)	0.497
CBN	C_{max} (μg/L)	37.3 (16 – 476)	48.1 (6.8 – 138)	0.376
	t_{max} (h)	0.5 (0.5 – 0.5)	0.5 (0.5 – 2)	0.693
	$AUC_{0\rightarrow30h}$ (μg/L.h)	37.6 (10.4 – 266)	41.7 (8.5 – 179)	0.771
THCCOOH	C_{max} (ng/L)	126 (59.7 – 430)	24.4 (0 – 77.7)	>0.0001
	t_{max} (h)	1 (0.5 – 10.5)	2 (0 – 13.5)[2]	0.693
	$AUC_{0\rightarrow30h}$ (ng/L.h)	1206 (408.1 – 5816.1)	-	-

[1] $p > 0.05$ indicates no significant difference between the daily and occasional users

[2] 2 of 10 occasional smokers were never positive for THCCOOH