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Cannabinoid Disposition in Oral Fluid after Controlled Cannabis Smoking in Frequent and Occasional Smokers

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Abstract

Oral fluid (OF) is an increasingly popular alternative matrix for drug testing, with cannabinoids being the most commonly identified illicit drug. Quantification of multiple OF cannabinoids and understanding differences in OF cannabinoid pharmacokinetics between frequent and occasional smokers improves test interpretation. The new Oral-Eze[®] OF collection device has an elution buffer that stabilizes analytes and improves drug recovery from the collection pad; however, its performance has not been independently evaluated. After controlled smoking of a 6.8% 9-tetrahydrocannabinol (THC) cannabis cigarette by frequent and occasional smokers, OF was collected with the Oral-Eze device for up to 30h. Samples were analyzed for multiple cannabinoids by a validated 2D-GC-MS method. Frequent smokers had significantly greater OF THCCOOH concentrations than occasional smokers at all times, and showed positive results for a significantly longer time. We evaluated multiple cannabinoid cutoffs; the shortest last detection times were observed when THC 1µg/L, and CBD or CBN 1µg/L. With these cutoffs, last detection times, about 1–13.5h, were not significantly different between groups, demonstrating suitability for short-term cannabinoid detection independent of smoking history. Cutoffs utilizing THC alone or combined with THCCOOH showed significantly different last detection times between groups. The widest detection windows were observed with THC 1 or 2µg/L or THCCOOH 20ng/L. Our data illustrate the effectiveness of the Oral-Eze device for OF collection, the impact of self-administered smoked cannabis history on OF cannabinoid results, and the ability to improve interpretation and tailor OF cannabinoid cutoffs to fulfill the detection window needs of a given program.

Keywords

oral fluid; Oral-Eze; cannabinoids

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Introduction

Oral fluid (OF) is an increasingly popular matrix in drug testing programs. OF collection is less invasive, does not require same-sex collectors, and minimizes sample adulteration, dilution and substitution than urine^[1]. Also, OF concentrations more closely correlate with blood than urine concentrations^[2–5], making it an attractive matrix for estimates of impairment and time since prior drug administration. OF drug detection windows are dependent upon many factors, including the drug and/or metabolites monitored, cutoffs selected, drug administration route, collection method, and amount and frequency of drug exposure. Programs focusing on recent use and impairment, (e.g. the European Union's Driving Under the Influence of Drugs, Alcohol and Medicines [DRUID] program and accident investigations) are interested in short detection times to better correlate with performance impairment^[2, 6]. Workplace and pain management programs prefer long detection times because testing intervals are widely separated. Treatment programs, which test patients twice or thrice weekly for compliance and relapse, prefer intermediate detection times.

Cannabis was the most commonly used illicit substance in the U.S. and globally in 2011–2012^[7–8] and the most common illicit substance in U.S. drivers testing positive for potentially impairing drugs in 2007^[9]. Therefore, cannabis detection is an important component of clinical, forensic, and driving under the influence of drugs (DUID) testing programs. The primary psychoactive component, ⁹-tetrahydrocannabinol (THC), contaminates the oral mucosa during smoking^[3] and can be detected 24–72h after last smoking, depending upon cutoffs applied^[3, 10–12]. OF THC concentrations are initially high after smoking, but quickly drop within 1–3h^[3, 13–15]. The phase I metabolite 11-hydroxy-THC (11-OH-THC) is not usually detected or is present at low concentrations (0.3–1.3µg/L) in OF after controlled smoked or oral cannabis. Maximum 11-nor-9-carboxy-THC (THCCOOH) concentrations after smoking were 134–760ng/L in OF collected with the Quantisal™ (Immualysis) device^[15, 19], and 560ng/L in expectorated OF^[18]. While peak THC concentrations occur immediately after smoking, peak THCCOOH concentrations typically occur 1–2h after smoking^[15]. THCCOOH has an extended window of detection compared to THC in frequent smokers^[11, 15]. Cannabinol (CBN) and cannabidiol (CBD) are minor, naturally occurring cannabinoids, with maximum CBN and CBD concentrations of 2.6–588 and 4.8–1558µg/L, respectively. Both analytes could be detected 2–6h after smoking^[15].

Quantification of multiple OF cannabinoids improves test interpretation, as each has a different detection window, mechanism of entry into OF, and possibly different profiles in frequent and occasional cannabis smokers. Additionally, it is unclear if OF collected with various commercially available devices, containing different elution and stabilization buffers, will have similar cannabinoid profiles over time. The Substance Abuse and Mental Health Services Administration (SAMHSA) proposed a 4µg/L THC OF screening cutoff and a 2µg/L THC OF confirmation cutoff^[20].

The present study quantified OF THC, CBD, CBN, 11-OH-THC, and THCCOOH collected with the Oral-Eze device from 14 frequent and 10 occasional cannabis smokers for up to 30h

after controlled smoking of a single 6.8% THC cigarette. To the best of our knowledge, this is the first performance evaluation of this new OF collection device after controlled cannabis smoking. We characterize and contrast time courses of these cannabinoids in OF from two different populations of cannabis smokers, evaluate detection windows with proposed and alternative cutoffs, and suggest combinations of cannabinoid cutoffs that might reflect different drug detection windows in OF collected with the Oral-Eze device. These controlled human smoked cannabis administration data will improve OF cannabinoid interpretation in clinical, drug treatment, law enforcement, anti-doping and workplace drug testing programs.

Materials and Methods

Participants

Frequent and occasional cannabis smokers (age 18–45 years) were recruited from the community by advertising and word-of-mouth. Frequent and occasional smokers were required to have an average smoking frequency of ≥ 4 times per week or <2 times per week in the past 3 months, respectively. Additional inclusion criteria were peripheral veins suitable for venipuncture, blood pressure ≥ 140 mmHg systolic and 90 mmHg diastolic, heart rate ≥ 90 bpm, and an electrocardiogram with 3-min rhythm strip without clinically significant abnormalities. Exclusion criteria included history of clinically significant illness, based on medical history, physical examination, and clinical laboratory tests; history of a clinically significant adverse event associated with cannabis intoxication; donation of more than 450 mL blood in previous 30 days; pregnancy or nursing; or interest or participation in drug abuse treatment within the past 60 days. The National Institute on Drug Abuse (NIDA) Institutional Review Board approved the study. All participants provided written informed consent.

Smoked Cannabis Administration

Participants resided on a closed residential unit the night prior to and following drug administration. Standardized cannabis cigarettes, supplied by the NIDA Chemistry and Physiological Systems Research Branch, contained approximately (mean $\pm$ standard deviation) $6.8 \pm 0.2\%$ THC (54 ± 2 mg), $0.25 \pm 0.08\%$ CBD (2.0 ± 0.6 mg), and $0.21 \pm 0.02\%$ CBN (1.7 ± 0.2 mg). Participants smoked a single cigarette *ad libitum* for up to 10 min while seated in a quiet room.

OF Collection and Analysis

OF was collected with the Oral-Eze[®] device (Quest Diagnostic[®], Madison, NJ) at admission (16–19 h before drug administration), 1 h before, and 0.5, 1, 2, 3, 4, 5, 6, 8, 10.5, 13.5, 21, 24, 26, 28, and 30 h after drug administration. Oral intake, including smoking, was prohibited 10 min before OF collection. The collection device consists of an absorptive cotton pad, a volume adequacy indicator that turns blue upon collection of 1 mL OF, and a plastic tube containing 2 mL stabilizing buffer, yielding a 1:3 OF dilution. Following manufacturer's recommendations, the collection pad resided in the stabilizing buffer at room temperature for ≥ 12 h to allow analyte elution from the pad. OF samples were generally analyzed within 24 h of collection, except 5 within 96 h. Low-volume specimens are recorded at the time of collection and analyzed as collected, without applying weight corrections.

We quantified THC, CBD, CNB, 11-OH-THC, and THCCOOH in OF by a previously published two-dimensional gas chromatography-mass spectrometry (2D-GC-MS) method^[21], with the following modifications: a) calibrators and quality controls (QC's) were prepared with 0.25mL blank authentic OF and 0.5mL Oral-Eze buffer (0.75mL analyzed), b) a positive pressure manifold replaced vacuum for solid phase extraction, c) before loading the first elution solvent, 0.4mL hexane was added to prime the solid phase extraction columns, d) the GC column configuration was reversed, with the DB-1MS column as primary and the ZB-50 as the secondary column, e) THCCOOH LOQ was increased to 15ng/L (upper LOQ 500ng/L), f) THCCOOH low and medium QCs were 45 and 120ng/L, respectively, and the high QC remained 300ng/L. Linear dynamic ranges for the other analytes were: 0.5–50µg/L for THC, 11-OH-THC, and CBD and 1.0–50µg/L for CBN. Intra-assay imprecision was 1.0–4.7%, inter-assay imprecision was <7.6%, and bias was 88.2–110.1%. Cannabinoid recoveries from the pad were THC 42.5–48.8%, CBD 33.5–47.7%, CBN 35.6–58.7%, 11-OH-THC 43.5–54.5% and THCCOOH 68.1–86.2%. Extraction efficiencies observed during validation for d₀-analytes and d₃-internal standards ranged from 58.6–126.6%.

Data Analysis

Statistical evaluation was performed with IBM SPSS Statistics version 20 for Windows (Armonk, NY) and Microsoft Excel 2007. Group medians were compared with Mann-Whitney exact *U*-test. When calculating statistics, concentrations <LOQ were set to 0, and medians >30h were set to 30. Differences in maximum observed concentrations ($C_{\max}$) and times to $C_{\max}$ ($t_{\max}$) were calculated with concentrations observed after dosing. Area under the curve (AUC) was calculated for each participant from –1 to 30h by the linear trapezoidal method and medians compared for each analyte. We evaluated three smoking history parameters on cannabinoid concentrations at 0.5h by Spearman's rank-order correlation. For frequent smokers, h between last smoking and admission were divided by 24 to get days since last smoking, and daily smoking frequency was multiplied by 30 to give monthly smoking frequency; data from all participants were evaluated simultaneously (not separated by group). Single and multi-analyte cutoffs were evaluated based on our previous work^[11, 15]. Survival curves based on these cutoffs were constructed via the Kaplan-Meier method and differences evaluated by the log-rank test; the conditional probability for producing a positive OF sample at time *t* was estimated by the product of positivity probability at time *t* and positivity probability beyond *t* among those positive at time *t*. If a participant did not produce a negative OF sample by their last collection, their data were censored. Survival curves were not constructed for cutoffs where all participants' data in a group were censored. Unless otherwise specified, statistical significance was present if $p < 0.05$.

Results

Fourteen frequent (10 males, 4 females; ages 19–37 years) and ten occasional (7 males, 3 females; ages 23–41 years) cannabis smokers were admitted to the closed residential unit up to 19h before dosing. Frequent and occasional smokers reported mean (SD) cannabis smoking for 13.3 ± 1.1 and 1.3 ± 1.6 of 14 days before admission, respectively. Age of first

cannabis smoking was 15.1 ± 3.5 and 15.4 ± 2.1 years, respectively. Participants M and N were recruited as occasional smokers based on self report; however, their biological cannabinoid concentrations in blood, plasma, urine, and OF warranted reclassification as frequent smokers, with inclusion of their data in that group. Additional demographic data are presented in Table 1.

Participants provided 404 OF samples (234 frequent, 170 occasional). Low OF volume collections (time) occurred in 1 (–1h), 10 (0.5h), 7 (1h), 1 (2h), and 2 (5h) frequent smokers' samples according to the Oral-Eze volume indicator. For occasional smokers, low-volume collections occurred in 9 (0.5h), 8 (1h), 6 (2h), 3 (3h), and 1 (6h) samples.

Median (interquartile range) concentration-time curves for THC, CBD, CBN, and THCCOOH are presented in Figure 1. When comparing group median concentrations at every timepoint, a Bonferroni correction was implemented to reduce Type I errors that may occur with multiple comparisons; therefore, significant differences between concentrations were present if $p < 0.003$. Table 2 summarizes % positive OF samples for these analytes. Median THC concentrations at admission and baseline were significantly different between groups. After smoking, there were no significant differences between median THC concentrations for frequent and occasional smokers at any timepoint throughout 30h. Frequent smokers' OF THC concentrations were above LOQ ($0.5 \mu\text{g/L}$) in 13 samples collected at admission (92.9%, range $3.2\text{--}236.8 \mu\text{g/L}$) and in 12 samples (85.7%, range $0.8\text{--}112 \mu\text{g/L}$) at baseline (–1h); no THC was detected in any occasional smoker's OF preceding cannabis smoking. THC detection rates for both groups were similar from 0.5–21h (90–100%). THC was detected in 67% of frequent and 10% of occasional smokers' OF 30h after smoking.

CBD concentrations were not significantly different between groups at any time. Three frequent smokers (21%) had CBD-positive OF at admission, but all samples were negative at baseline. CBD detection rates between groups were similar and no participant had CBD-positive OF after 6h. Frequent smokers had significantly higher OF CBN at admission compared to occasional smokers. Ten frequent smokers' OF were CBN-positive upon admission (71%), including the three CBD-positive participants; none were CBN-positive at baseline. CBN detection rates after smoking were generally similar between groups, with no frequent or occasional smoker producing CBN-positive OF after 10.5 or 13.5h, respectively. In general, maximum CBN concentrations were approximately twice those of CBD.

11-OH-THC was quantified in five frequent and three occasional smokers' OF samples, never exceeding $1.2 \mu\text{g/L}$. Two frequent and one occasional smoker had two 11-OH-THC-positive samples at 0.5 and 1h post-dose. The other three frequent and two occasional smokers had one 11-OH-THC-positive OF sample at 0.5h. OF THCCOOH concentrations were significantly greater in frequent than occasional smokers at every timepoint. Additionally, all frequent smokers produced THCCOOH-positive OF samples from admission to 30h post-smoking. THCCOOH detection rates in occasional smokers peaked at 90% at 2h (range $0\text{--}152.4 \text{ng/L}$). THCCOOH was observed in 10% of occasional smokers 28h after smoking.

Table 3 compares median $C_{\max}$, $t_{\max}$, concentrations of last positive samples (C_{last}), times of C_{last} (t_{last}), and $AUC_{-1 \rightarrow 30h}$ for THC, CBD, CBN and THCCOOH between groups. The first post-dose collection at 0.5h was missed for participant C; these data were not included in $C_{\max}$ and $t_{\max}$ determinations. Participant B left before the 28 and 30h collections, and participant E's 30h sample was broken; hence, these data were excluded for C_{last} and t_{last} . In addition, these three participants' data were excluded from $AUC_{-1 \rightarrow 30h}$ calculations.

One frequent smoker (7%) had an OF THC concentration before dosing greater than the post-dose THC $C_{\max}$. Frequent smokers had a significantly earlier THC $t_{\max}$ and later THC t_{last} than occasional smokers over 30h. Frequent smokers also had significantly earlier CBD and CBN $t_{\max}$ than occasional smokers. Five frequent smokers (36%) had higher OF THCCOOH concentrations on admission than after cannabis smoking, reflecting previous self-administered cannabis. Significantly higher THCCOOH $C_{\max}$, C_{last} , and later t_{last} were observed in frequent smokers. There were no significant differences in $AUC_{-1 \rightarrow 30h}$ for THC, CBD and CBN, but THCCOOH $AUC_{-1 \rightarrow 30h}$ was significantly greater in frequent than occasional smokers.

No significant correlations were observed between years smoked, days since last cannabis smoking, or number of joints smoked per day and THC, CBD, or CBN 0.5h OF concentrations; however, days since last cannabis smoking ($\rho = -0.626$, $p=0.002$) and joints smoked per month ($\rho=0.702$, $p=0.001$) were significantly correlated with 0.5h THCCOOH concentrations.

Figure 2 depicts last positive OF detection times for various cutoffs. At the SAMHSA proposed THC $2\mu\text{g/L}$ confirmation cutoff, median times of frequent and occasional smokers' last positive OF sample were 21 (6–30)h, and 13.5 (5–26)h, respectively. At the THC $1\mu\text{g/L}$ cutoff, frequent and occasional smokers' median last positive OF times were 28 (10.5–30)h and 21 (8–28)h, respectively. With a 20ng/L THCCOOH cutoff, all frequent smokers' OF samples were positive at last collection (26–30h), while occasional smokers' median time for last positive THCCOOH OF sample was 8 (1–21)h.

A combined THC 1 or $2\mu\text{g/L}$ and THCCOOH 20ng/L cutoff did not change last detection times in frequent smokers' OF compared to THC alone, as all participants' samples were positive for THCCOOH at their last collection time; occasional smokers' median last detection times were reduced to 8 or 6.5h when the THC component was 1 or $2\mu\text{g/L}$, respectively, (range for both cutoffs was 1–13.5h) when compared to THC alone. Adding CBN $1\mu\text{g/L}$ to THC $1\mu\text{g/L}$ shortened frequent and occasional smokers' median last detection times to 6(1–10.5)h and 5.5(2–13.5)h, respectively, compared to THC 1 or $2\mu\text{g/L}$ alone. Similarly, at THC and CBD $1\mu\text{g/L}$, frequent and occasional smokers' median last detection times were shortened to 3 and 2.5h, respectively (range 1–6h for both). Last detection times for the latter two cutoffs did not change if the THC limit was increased to $2\mu\text{g/L}$ (data not shown). Median last detection times did not change in frequent smokers with THC 1 or $2\mu\text{g/L}$ or THCCOOH 20ng/L , when compared to THCCOOH alone, because all frequent smokers' OF samples were positive for THCCOOH at their final collection, or in occasional smokers when compared to THC alone.

Figure 3 depicts detection rates at all collection times based on the 9 presented cutoffs. At THC 1 or 2µg/L, frequent smokers generally had greater detection rates than occasional smokers. Detection rates with THCCOOH 20µg/L were greater for frequent smokers throughout the study. With a THC 1 or 2µg/L *and* THCCOOH 20µg/L, frequent smokers' detection rates were not affected when compared to THC alone, but occasional smokers' detection rates decreased. By changing the cutoff to THC *or* THCCOOH instead of THC *and* THCCOOH, detection rates were maximized in both groups for the longest time. In contrast, addition of CBN or CBD to the THC criteria reduced detection rates and decreased last detection times.

Survival analyses based on the above cutoffs are presented in Figure 4. Frequent smokers had significantly longer detection times with the DRUID, SAMHSA, and THC 1 or 2µg/L *and* THCCOOH 20ng/L cutoffs than occasional smokers, but not with the THC 1µg/L and CBN or CBD 1µg/L cutoffs. Survival curves were not prepared for the THCCOOH 20ng/L, or THC 1 or 2µg/L *or* THCCOOH 20ng/L cutoffs because all frequent smokers' data were censored.

Discussion

SAMHSA currently suggests that low-volume OF collections be discarded and new samples collected. Our data showed that THC was identified in all low-volume Oral-Eze samples collected after dosing, although concentrations were artificially reduced due to greater dilution. This agrees with previously reported data showing increased positivity rates when low-volume collections were analyzed^[22].

Cannabinoid adsorption onto the pad can be problematic^[23]. Buffers aid drug elution from the pad, but may produce undesirable matrix effects, potentially requiring additional sample preparation. Analyte recoveries in the present study ranged from 33.5–86.2%. Lower recoveries occurred with the more lipophilic THC, CBN and CBD, and better recoveries with the more polar 11-OH-THC and THCCOOH. These recoveries were evaluated independently and differed from manufacturer's results.

Median (range) OF THC concentrations were 212 (40.0–6362), 287 (18.9–2440), 94.1 (16.0–519), and 2.1 (0.5–5.5)µg/L at 0.5, 1, 2, and 22h post dose, respectively, in Quantisal-collected samples from different frequent users after smoking a 6.8% cannabis cigarette^[15]. THC concentrations in expectorated OF in these participants after smoking the same potency cannabis cigarette were 527 (101–1524), 282 (35.4–1030), 31.7 (11.6–1310), and 1.8 (0.4–10.3)µg/L at the same timepoints, respectively^[18]. In the present study, OF THC concentrations in frequent smokers at 0.5, 1, 2, and 21h post dose were 803 (135–4146), 228 (36.9–969), 68.6 (10.9–267) and 4.6 (0–8.8)µg/L, respectively, agreeing with prior results.

These are the first OF cannabinoid data after controlled cannabis smoking in frequent and occasional cannabis smokers with the Oral-Eze collection device. Toennes et al. measured OF THC with the Intercept DOA Oral Specimen Collection Device (OraSure Technologies) in frequent and occasional smokers for 8h after smoking a 500 µg THC/kg cannabis cigarette^[13]. There were significant differences in median (range) THC C_{max} 5 min after

smoking in frequent and occasional smokers, 6,202 (387–71,147) and 1,242 (397–6,438)ng/g, respectively. Differences in THC $C_{\max}$ in the present study may have been missed because the first OF collection occurred 0.5h after smoking (the true $C_{\max}$ most likely occurred near the end of smoking), and THC concentrations were highly variable. It is unlikely OF would be collected so close to smoking in drug treatment, law enforcement, anti-doping or workplace drug testing programs.

In contrast, frequent smokers' THCCOOH concentrations were significantly higher than those of occasional smokers at all times, and THCCOOH was detected in frequent smokers for significantly longer. This is from frequent smokers' increased prior cannabis exposure, and greater THC-accumulation and release from body stores after admission^[11, 24]. This is supported by frequent smokers' significantly greater $AUC_{-1 \rightarrow 30h}$, as all frequent smokers' OF was THCCOOH-positive at –1h.

Days since last smoking and number of joints smoked per month (last three months) significantly correlated with THCCOOH 0.5h OF concentrations; lifetime years of smoking (which may not be related to smoking frequency), did not. THCCOOH is a metabolite with concentrations depending on parent THC body burden and prolonged release, a more important factor for frequent cannabis smokers. These results are supported by Lee et al.^[15], who showed that participants with <10 years lifetime smoking did not have significantly different THCCOOH $C_{\max}$ than those from participants with >10 years lifetime smoking.

Our evaluation of single and multiple cannabinoid cutoffs illustrate the potential for tailoring cutoffs to specific drug testing program needs. Short last detection times in both groups were obtained when CBD or CBN was added to a THC 1 μ g/L; this cutoff may be useful for DUID and accident investigations because the median last detection times fall within reported impairment windows^[2, 25]. CBD/CBN detection times at these cutoffs could be longer if the smoked cannabis contained greater CBD or CBN concentrations than were administered in the current study. Survival analyses also demonstrated that there were no significant differences between groups in the probability of being positive at a particular time, demonstrating their utility as smoking history-independent cutoffs. Intermediate to long-term last detection times were observed with THC 1 or 2 μ g/L cutoffs, and when THCCOOH 20ng/L was added to the THC component. Survival analyses showed that all four cutoffs, THC components alone and each combined with THCCOOH, were significantly different between groups. The combined THC *and* THCCOOH cutoffs did not change last detection times in frequent smokers when compared to THC alone, but lowered the median last detection time in occasional smokers to 8h. Longest last detection times for both groups were with the THC *or* THCCOOH cutoffs, which allowed either analyte to control the final detection time.

However, THC presence does not rule out acute passive environmental contamination. Moore et al. reported a maximum 17 μ g/L OF THC in a passive inhaler exposed to cannabis smoke for 3h in a Dutch coffee shop^[26]. Addition of THCCOOH 20ng/L to the THC cutoff protects against passive exposure, as it is not contained in cannabis smoke and no THCCOOH was found in OF collected from passive inhalers. Additionally, after oral THC administration, THCCOOH OF concentrations mirrored plasma THCCOOH concentrations,

while THC decreased over time after admission and was not quantifiable after the 37th 20 mg oral dose over 8 days^[17]. THC in OF after oral THC administration reflected previously self-administered smoked cannabis.

We demonstrated that the Oral-Eze[®] collection device was effective for OF cannabinoids monitoring in frequent and occasional smokers. We also demonstrated the value of monitoring THC, CBD, CBN and THCCOOH to improve interpretation of OF cannabinoid results. We showed that OF THCCOOH timecourse in frequent smokers is significantly different than that in occasional smokers, illustrating that smoking history alters cannabinoid concentrations over time. Finally, we suggested different cannabinoid OF cutoffs to meet the goals of different drug testing programs.

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Figure 1.

Median and interquartile range concentration-time curves for tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), and 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) in OF from frequent and occasional cannabis smokers after smoking one 6.8% THC cigarette. THC concentrations were log-transformed and values <LOQ were assigned a value of 0.25 (1/2 LOQ) for transformation purposes. The time-scales for CBD and CBN were truncated at the first time the upper quartile for both groups reached 0 (i.e. all data beyond that point were <LOQ). *indicates a significant difference in concentration at each timepoint between groups ($p < 0.003$)

Figure 2.

Last positive detection times for frequent and occasional smokers according to various cutoffs. Filled symbols represent last positive sample was not the last collected sample, empty symbols represent last collected sample = last positive sample.

Figure 3.
Detection rates (% positive) based on 9 different cutoffs in frequent and occasional smokers at every collection time.

Figure 4.

Probability of last positive sample with Oral-Eze[®] determined by survival analysis.

Censored cases were those without defined last positive samples (empty symbols in Fig. 2).

Table 1
Demographic characteristics and self-reported cannabis use histories for frequent and occasional cannabis smokers.

Frequent Smokers									
Smoking History									
Participant	Sex	Race ^a	Age, years ^b	BMI, kg/m ^{2b}	Age 1st Use, years ^b	Hours between last smoke & admission ^c	Days smoked in last 14 ^c	Joints smoked per day ^c	Lifetime years smoking ^c
A	M	AA	29.6	27.6	12	7.4	11	4	17.6
B	M	AA	19.4	22.6	15	4.3	13	5	4.4
C	M	AA	22.6	31.4	14	5.1	12	3	8.6
D	M	W	25.5	23.0	13	3.9	14	20	12.5
E	F	AA	19.9	32.4	11	2.6	14	3.5	8.9
F	M	AA	24.2	27.4	13	23.2	12	1.5	11.2
G	F	W	22.9	24.8	16	17.2	14	6	6.9
H	M	AA	37.3	23.0	25	1.6	14	3	12.3
I	F	AA	27.6	35.4	18	2.4	14	4	9.6
J	F	AA	26.9	20.4	14	3.8	14	21	12.9
K	M	AA	23.4	24.3	19	1.2	14	6	4.4
L	M	AA	28.7	28.1	14	9.5	14	6	14.7
M	M	AA	28.0	19.4	14	67.4 ^d	2 ^d	0.1 ^d	14.0
N	M	AA	23.8	30.7	14	273 ^d	1 ^d	0.1 ^d	9.8
Mean			25.7	26.4	15.1	6.9	13.3	6.9	10.6
Median			24.8	26.1	14.0	4.1	14.0	4.5	10.5
SD			4.6	4.8	3.5	6.8	1.1	6.5	3.8

Occasional Smokers									
Smoking History									
Participant	Sex	Race	Age, years	BMI, kg/m ²	Age 1st Use, years	Days between last smoke & admission	Days smoked in last 14	Joints smoked per month	Lifetime years smoking
O	M	W	25.6	29.4	16	16	0	2	9.6
P	M	W	25.4	23.7	13	31	0	2	12.4
Q	M	W	23.7	24.1	16	10	2	7	7.7

Occasional Smokers									
Smoking History									
Participant	Sex	Race	Age, years	BMI, kg/m ²	Age 1st Use, years	Days between last smoke & admission	Days smoked in last 14	Joints smoked per month	Lifetime years smoking
R	M	AA	38.2	21.0	19	2	2	2	19.2
S	M	Mixed	41.3	22.0	16	7	5	10	25.3
T	F	U	34.9	31.7	13	9	1	2	21.9
U	F	AA	36.5	47.8	18	2	2	4	18.5
V	M	Mixed	22.5	25.2	13	86	0	6	9.5
W	F	W	34.2	26.6	14	3	1	0.25	20.2
X	M	AA	31.7	21.8	16	18	0	8	15.7
Mean			31.4	27.3	15.4	18.4	1.3	4.3	16.0
Median			33.0	24.7	16.0	9.5	1.0	3.0	17.1
SD			6.7	8.0	2.1	25.4	1.6	3.2	6.0

^aW = White, AA = African American, U =unknown

^bData collected at screening for both groups

^cData collected at study session admission for both groups

^dSelf-reported data not consistent with biological sample cannabinoid concentrations, excluded from mean and median.

Table 2

Detection rates (% positive samples >LOQ) for ⁹-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), and 11-nor-9-carboxy-THC (THCCOOH) in frequent (N=14) and occasional smokers (N=10), except where noted. LOQ was 0.5µg/L for THC and CBD, 1µg/L for CBN, and 15ng/L for THCCOOH.

Time After Smoking (h)	Detection Rates (% Positive Samples)							
	THC		CBD		CBN		THCCOOH	
	Frequent	Occasional	Frequent	Occasional	Frequent	Occasional	Frequent	Occasional
-19	93	0	21	0	71	0	100	0
-1	86	0	0	0	0	0	100	0
0.5 ^a	100	100	100	100	100	100	100	30
1	100	100	100	100	100	100	100	50
2	100	100	86	90	93	100	100	90
3	100	100	36	40	71	80	100	70
4	100	100	43	20	71	90	100	80
5	100	100	7	20	57	50	100	60
6	100	100	7	10	50	50	100	60
8	100	100	0	0	7	30	100	60
10.5	100	90	0	0	7	10	100	30
13.5	100	100	0	0	0	10	100	30
21	93	90	0	0	0	0	100	20
24	79	40	0	0	0	0	100	10
26	79	40	0	0	0	0	100	10
28 ^a	62	40	0	0	0	0	100	10
30 ^b	67	10	0	0	0	0	100	0

^a N = 13 frequent smokers

^b N = 12 frequent smokers

Table 3

Summary statistics for median tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), and 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) pharmacokinetic parameters.

Analyte (LOQ)	Pharmacokinetic Parameter	Frequent smokers' median (range) ^a	Occasional smokers' median (range) ^b	Significance ^c
THC (0.5 µg/L)	C _{max} , µg/L	803 (135–4146)	337 (93.3–2760)	0.446
	t _{max} , h	0.5	0.8 (0.5–2)	0.007
	C _{last} , µg/L	1.1 (0.7–2.9)	0.9 (0.6–1.5)	0.088
	t _{last} , h	>30 (13.5–>30)	22.5 (13.5–30)	0.045
	AUC _{-1→30h} , ng·h/mL	777 (258–5498)	757 (254–4785)	0.705
CBD (0.5 µg/L)	C _{max} , µg/L	14.8 (1.4–162)	7 (1.9–111)	0.384
	t _{max} , h	0.5 (0.5–1)	1 (0.5–2)	0.015
	C _{last} , µg/L	1.7 (1.3–7.8)	1.8 (1.0–6.7)	0.859
	t _{last} , h	2 (1–6)	2.5 (1–6)	0.482
	AUC _{-1→30h} , ng·h/mL	29.0 (4.7–211)	11.6 (4.1–185)	0.468
CBN (1 µg/L)	C _{max} , µg/L	32.2 (3.3–284)	34 (7.5–255)	0.693
	t _{max} , h	0.5 (0.5–1)	0.8 (0.5–2)	0.007
	C _{last} , µg/L	1.5 (1.1–4.6)	1.5 (1–2.4)	0.592
	t _{last} , h	6 (1–10.5)	5.5 (2–13.5)	0.583
	AUC _{-1→30h} , ng·h/mL	40.6 (15.8–364)	60.7 (12.2–410)	0.756
THCCOOH (15 ng/L)	C _{max} , ng/L	168 (70.0–1107)	39.6 (20.9–168.4)	<0.001
	t _{max} , h	2 (0.5–30)	2 (0.5–3)	0.791
	C _{last} , ng/L	64.9 (25.3–191)	21.2 (15.5–39.7)	<0.001
	t _{last} , h	>30 (26–>30)	8 (1–28)	<0.001
	AUC _{-1→30h} , pg·h/mL	3332 (1236–8218)	180 (20.9–842)	<0.001

^aN = 13 for C_{max} and t_{max}; 12 for C_{last} and t_{last}; and 11 for AUC_{-1→30h}

^bN = 10 for all parameters

^cP-values in **bold** denote significance (<0.05)