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Cannabinoids in Oral Fluid by on-site immunoassay and by GC-MS using two different oral fluid collection devices

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Abstract

Oral fluid (OF) offers non-invasive sample collection for on-site drug testing, but performance of on-site tests with occasional and frequent smokers' OF to identify cannabinoid intake requires further evaluation. Furthermore, to our knowledge, no studies evaluate differences in cannabinoid disposition among OF collection devices with authentic OF samples after controlled cannabis administration.

14 frequent (4x/week) and 10 occasional (<2x/week) adult cannabis smokers smoked one 6.8% ⁹-tetrahydrocannabinol (THC) cigarette *ad libitum* over 10 min. OF was collected with the StatSure Saliva Sampler™, Oral-Eze®, and Draeger DrugTest® 5000 test cassette before and up to 30h after cannabis smoking. Test cassettes were analyzed within 15 min and gas chromatography-mass spectrometry cannabinoid results obtained within 24h. Cannabinoid concentrations with the StatSure and Oral-Eze devices were compared and last cannabinoid detection times (*t*_{last}) and DrugTest 5000 test performance were assessed at different cannabinoid cutoffs.

11-nor-9-carboxy-THC (THCCOOH) and cannabinol concentrations were significantly higher in Oral-Eze than Stat-Sure samples. DrugTest 5000 *t*_{last} for a positive cannabinoid test were median (range) 12h (4-24h) and 21h (1-30h) for occasional and frequent smokers, respectively. Detection windows in screening and confirmatory tests were generally shorter in occasional than frequent smokers, especially when including THCCOOH 20ng/L in confirmation criteria. No differences in *t*_{last} were observed between collection devices, except for THC 2μg/L.

We report significant differences in THCCOOH and cannabinol, but not THC, concentrations between OF collection devices, which may influence OF data interpretation. The DrugTest 5000 on-site device had high diagnostic sensitivity, specificity and efficiency for cannabinoids.

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Keywords

Cannabinoid; Oral Fluid; On-site test; Draeger DrugTest 5000

Introduction

Cannabis is the most commonly used illicit drug worldwide, with 2.8-5.0% (129-230 million people) of 15-64 year-olds consuming cannabis at least once in 2011 [1].

⁹-Tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis, was the most prevalent illicit drug detected in injured drivers in Victoria, Australia (9.8%) [2]. Cannabinoids were found in 8.6% of nighttime drivers' blood and/or oral fluid (OF) in the 2007 US Roadside Survey [3].

With changes to drug policy for recreational and medicinal cannabis intake, increased recreational and medicinal cannabis use [1,4-5] and decreased perceived risk [3], rapid and accurate on-site cannabinoid testing is required. OF is increasingly utilized for on-site drug testing in drug treatment, workplace, pain management, and driving under the influence of drugs (DUID) testing. OF advantages include non-invasiveness, observed specimen collection, and lower potential for adulteration compared to urine. Optimal OF on-site devices require rapid analysis times, OF collection volume indicators, objective result interpretation, and quality control parameters such as adequacy of lateral flow. However, differences in device formulation including elution buffers, sample collection volume, buffer dilution, cannabinoid antibodies, and detection mechanisms make performance comparison among devices difficult. Furthermore, manufacturers make frequent device reformulations to improve cannabinoid sensitivity. Many previously available on-site devices failed to meet the 80% diagnostic sensitivity, specificity, and efficiency criteria proposed by the Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) project for OF drug detection [6-15]. On-site OF cannabinoid efficiencies were 80% for OraLine® IV s.a.t. [8], 57.5-92.8% for Drugwipe 5 [10-13,16], 60-94.3% for Cozart DDSV [14,12,17], 66-93.0% for RapidStat® [12,15,13,17,16], 94.3% for A-Concateno DDS® [16], 68-96.1% for DrugTest 5000® [12-13,17,16], and 78-90.9% for OrAlert™ [12,17]. Although the OraLine IV s.a.t. and A-Concateno DDS devices achieved 80% efficiency, sensitivity was only 69.2% and 37.8%, respectively. Overall, the authors concluded that the DrugTest 5000 achieved the best diagnostic efficiency for OF cannabinoids detection [13,17,16,18].

We recently documented high diagnostic sensitivity (90.7%), specificity (75.0%), and efficiency (87.9%) for the DrugTest 5000 with the manufacturer's 5 µg/L screening cutoff and a 2 µg/L THC confirmation cutoff [19]. However, that study included only frequent smokers and monitored cannabinoids for only 22h after smoking, leading to overall high cannabinoid concentrations and too few true negatives to evaluate specificity. Others also evaluated this newest DrugTest 5000 version (5 µg/L THC screening cutoff) and reported diagnostic sensitivity, specificity, and efficiency of 53-93, 71-99 and 84-94.3%, respectively, with OF THC confirmation cutoffs of 1-10 µg/L [12-13,16-17]. Recency of smoking was shown to impact diagnostic sensitivity and efficiency [12,18].

To our knowledge, no studies evaluate differences among OF collection devices following controlled smoking and with authentic OF samples, although significantly higher concentrations were found in expectorated OF from Dutch “Coffee Shop” patrons compared to samples consecutively collected from the same individuals with the StatSure device [20]. Furthermore, OF THC concentrations in consecutively collected duplicate expectorated samples were much more variable than samples collected with the StatSure device. No significant differences between simultaneously collected OF samples with the Quantisal [21] or Intercept devices [22] were noted.

To address these knowledge gaps, we evaluated performance characteristics and OF detection windows with the on-site DrugTest 5000 and the StatSure and Oral-Eze devices in occasional and frequent cannabis users following smoking of a 6.8% THC cigarette. THC, 11-nor-9-carboxy-THC (THCCOOH), 11-hydroxy-THC (11-OH-THC), cannabidiol (CBD), and cannabinol (CBN) were quantified by two-dimensional gas chromatography-mass spectrometry (2D-GC-MS) [23] to investigate different cannabinoid markers and windows of cannabinoid detection to meet the goals of diverse drug testing programs.

Methods

Participants

Healthy male and female cannabis smokers provided written informed consent to participate in this National Institute on Drug Abuse Intramural Research Program Institutional Review Board-approved study. Individuals were recruited by television, radio and newspaper advertisements, flyers, and participant referrals. Participants received a comprehensive medical and psychological evaluation to verify compliance with eligibility criteria. Inclusion criteria were ages 18 to 45 years and self-reported average frequency of cannabis smoked of less than twice per week (occasional smoker) or at least four times per week (frequent smoker) in the past 3 months. History of cannabis use was confirmed by a positive urine cannabinoid test for chronic frequent smokers. Exclusion criteria included breastfeeding or pregnant women; current clinically significant medical condition or history of neurological illness; history of a clinically significant adverse event associated with cannabis intoxication; >450mL blood donation within 30 days of drug administration; clinically significant anemia; increased systolic or diastolic blood pressure or heart rate >100 bpm after 5 min rest; clinically significant electrocardiogram abnormality; or interest in drug abuse treatment within 60 days of study screening. Pregnancy tests were administered at screening and on study admission to women with reproductive potential.

Study Design

Participants entered the secure research unit approximately 19h prior to smoking to preclude intoxication at the time of cannabis dosing. Participants smoked one (average $\pm$ SD) 6.8 $\pm$ 0.2% (54mg) THC, 0.25 $\pm$ 0.08 CBD (2mg), and 0.21 $\pm$ 0.02% CBN (1.6mg) cannabis cigarette *ad libitum* for up to 10min. OF was collected with the StatSure Saliva Sampler™ (StatSure Diagnostics Systems, Inc., Framingham, MA), followed by the Oral-Eze® (Quest Diagnostics, Madison, NJ) and the DrugTest 5000 (Draeger Safety Diagnostics, Lübeck, Germany). The StatSure device consists of an absorptive cellulose pad placed below the

tongue, a volume adequacy indicator that turns blue upon collection of 1.0mL OF, and a polypropylene tube containing 1mL elution/stabilizing buffer, yielding a 1:2 OF dilution. The Oral-Eze device has an absorptive cotton pad positioned between the lower cheek and gum (with plastic shield against the cheek), a volume adequacy indicator that turns blue upon collection of 1.0mL OF, and a plastic tube containing 2mL stabilizing buffer, yielding a 1:3 OF dilution. The DrugTest 5000 test cassette is equipped with a polymeric non-compressible pad for OF collection. OF was collected by swiping the test cassette on the tongue and side of the cheeks. The test cassette collects $270\mu\text{L}\pm 15\%$ OF, as indicated by the volume adequacy indicator. Oral intake (eating, drinking, cigarette smoking) was prohibited 10min before OF collection. Samples were collected on admission, 1h before, and 0.5, 1, 2, 3, 4, 5, 6, 8, 10.5, 13.5, 21, 24, 26, 28, and 30h after the start of smoking. OF was collected until the volume indicator turned blue or for a maximum of 10 min. StatSure samples were stored upright at 4°C and Oral-Eze samples stored horizontally at room temperature according to manufacturers' recommendations; all (except 5 Oral-Eze samples analyzed within 96h) were analyzed within 24h of collection. Participants remained on the secure residential unit until the end of the study.

Sample Analysis

THC, 11-OH-THC, THCCOOH, CBN, and CBD were quantified by 2D-GC-MS according to a previously published method [23], with minor modifications. For the StatSure, calibrators and quality controls were prepared in 0.25mL blank OF and 0.25mL StatSure buffer to account for OF dilution. For the Oral-Eze, calibrators and quality controls were prepared with 0.25mL blank OF and 0.5mL Oral-Eze buffer. The GC column configuration for neutral cannabinoid analysis was changed, with the DB-1MS (Agilent Technologies, Wilmington, DE) column as the primary and the ZB-50 (Phenomenex, Torrance, CA) as the secondary column. Before loading the first elution solvent, 0.4mL methanol (StatSure) or hexane (Oral-Eze) was added to the solid phase extraction columns. Limits of quantification (LOQ) were 0.5 $\mu\text{g/L}$ for THC, 11-OH-THC, and CBD; 0.5 $\mu\text{g/L}$ (StatSure) or 1 $\mu\text{g/L}$ (Oral-Eze) for CBN; and 15ng/L for THCCOOH. For StatSure, the linear range was 0.5-50 $\mu\text{g/L}$ (THC, CBD, CBN, and 11-OH-THC), and 10-500ng/L (THCCOOH), and for Oral-Eze, 0.5-50 $\mu\text{g/L}$ (THC, CBD, and 11-OH-THC), 1-50 $\mu\text{g/L}$ (CBN), and 15-500ng/L (THCCOOH). Intra-assay imprecision were 1.0-2.7% (n=6) and 1.0%-4.7% (n=6) for StatSure and Oral-Eze devices, respectively; inter-assay imprecision was <7.6% for both. OF specimens were diluted with drug free OF-buffer mixture if analyte concentrations exceeded the upper LOQ.

Data Analysis

Qualitative OF DrugTest 5000 cannabinoid results at the preprogrammed 5 $\mu\text{g/L}$ THC cutoff were evaluated against quantitative StatSure and Oral-Eze OF 2D-GC-MS results. True positive (TP, DrugTest 5000 and GC-MS positive), true negative (TN, DrugTest 5000 and GCMS negative), false positive (FP, positive DrugTest 5000, but negative GC-MS) and false negative (FN, negative DrugTest 5000, but positive GC-MS) results were calculated at DrugTest 5000 screening cutoffs of 5 $\mu\text{g/L}$ THC and GC-MS THC confirmation cutoffs of 1 $\mu\text{g/L}$ (DRUID), and 2 $\mu\text{g/L}$ (Substance Abuse and Mental Health Services Administration, SAMHSA), as well as confirmation cutoffs of THC and/or THCCOOH (20 ng/L).

Sensitivity ($100 \times (TP/[TP+FN])$), specificity ($100 \times (TN/[TN+FP])$), and efficiency ($100 \times ((TP+TN)/[TP+TN+FP+FN])$) were calculated at multiple confirmation cutoffs. Rates of detection and windows of detection were evaluated with the DrugTest 5000 screen and different confirmation analytes and cutoffs.

Mann-Whitney U test was utilized to compare time of last detection between occasional and frequent smokers at various screening and confirmation cutoffs; detection 30h was assigned as 30h for statistical purposes. Wilcoxon Signed Rank test was applied to compare Oral-Eze and StatSure concentrations and times of last detection (t_{last}) between devices; a 1 $\mu\text{g/L}$ cutoff was employed for CBN to keep consistency between devices. Any pair where the StatSure or Oral-Eze volume adequacy indicator did not turn blue was excluded. All analyses were done with SPSS Version 20 (IBM, Armonk, NY), with two-tailed $p < 0.05$ considered significant.

Results

Human Participants

Fourteen healthy frequent and 10 occasional smokers (17 men, 7 women), ages 19-41 years, participated in the study (Table 1). Frequent smokers were significantly younger, had smoked for a significantly shorter period of their lifetime, and smoked significantly more recently, more joints or joint-equivalents (empirically-normalized joint consumption, to account for different smoking methods [i.e. bowl, pipe, blunt]) in the last 14 days and more frequently compared to occasional smokers. Two participants (M and N) were originally classified as occasional smokers by self-report, but following analysis of multiple biological (blood, urine, and oral fluid) samples, were reclassified as chronic frequent smokers.

Sample Analysis

Participant B withdrew from the study after the 26h sample collection; 28 and 30h OF samples were not collected. Additional Oral-Eze and DrugTest 5000 samples were missed for participant C at 0.5h due to an adverse event, and for participant E at 30h due to a broken Oral-Eze tube. There were 404 DrugTest 5000-StatSure sample pairs and 403 DrugTest 5000-Oral-Eze sample pairs. Nine DrugTest 5000 samples (2.2%) produced invalid results, yielding 395 OF pairs for comparison with StatSure and 394 OF pairs for comparison with Oral-Eze. Quantitative cannabinoid disposition in OF collected with the StatSure and Oral-Eze devices were previously published [24-25].

StatSure and Oral-Eze Cannabinoid Concentration Comparison

StatSure and Oral-Eze THC and CBD concentrations were not significantly different between devices ($Z = -1.500$, $p = 0.134$ for THC and $Z = -1.551$, $p = 0.121$ for CBD) in consecutively collected samples. However, StatSure and Oral-Eze THCCOOH and CBN concentrations differed significantly by device ($Z = -11.439$, $p < 0.001$ for THCCOOH and $Z = -2.520$, $p < 0.05$ for CBN), with THCCOOH and CBN concentrations being generally higher in Oral-Eze samples. Given the low THCCOOH detection rate in occasional smokers [24-25], we also compared THCCOOH concentrations between devices for frequent smokers only; concentrations remained significantly different ($Z = -10.380$, $p < 0.001$).

Detection Rates and Windows of Detection

StatSure and Oral-Eze OF detection rates, with and without DrugTest 5000 results, for THC 1 and 2 µg/L, THCCOOH 20 ng/L, THC 2 µg/L or THCCOOH 20 ng/L, and THC 2 µg/L and THCCOOH 20 ng/L are shown in Figures 1 and 2. All occasional smokers were negative on admission for all analytes and cutoffs, while 85.7-100% of frequent smokers were positive on admission, depending on the collection device and cutoff. Adding the DrugTest 5000 results generally reduced detection rates (Figures 1 and 2).

Detection windows were generally shorter in occasional smokers compared to frequent smokers, especially when including THCCOOH 20 ng/L in the confirmation criteria (Table 2). Three frequent smokers were still DrugTest 5000 positive at 30h, but had 1, 5, and 5 negative samples prior to 30h. Cannabinoid t_{last} values were not significantly different between devices, except for THC 2 µg/L (Table 3).

DrugTest 5000 and Confirmation Comparison

Diagnostic sensitivity, specificity and efficiency at multiple confirmation cutoffs are shown in Table 4. When confirming for THC only, diagnostic sensitivity was 5.7-11.0 percentage points higher in frequent as compared to occasional smokers, due to longer detection windows and higher true positive rates (e.g., 70.6% vs. 59.6% in frequent and occasional smokers, respectively, at the 1 µg/L Oral-Eze cutoff). Similarly, increased diagnostic sensitivity, specificity, and efficiency were generally documented when considering only 6 or 8h post-smoking, due to higher concentrations and higher true positive rates in occasional and frequent smokers.

Discussion

To our knowledge, no other studies compare cannabinoid concentrations among OF collection devices from authentic OF samples after controlled cannabis smoking. In addition, we evaluated detection windows of one screening and two confirmation OF collection devices for cannabinoids in OF from occasional and frequent smokers.

THCCOOH and CBN concentrations were significantly higher in Oral-Eze devices. StatSure samples were collected immediately before Oral-Eze samples. Perhaps the presence of the StatSure device in the mouth stimulated OF excretion, but it is unclear how this could have resulted in higher THCCOOH and CBN concentrations with the Oral-Eze device. Although both devices theoretically collect 1 mL, actual OF volume collected might vary. Previous work documented (based on weight) a mean (RSD) of 0.952 mL (11.97%) OF for OF collected with the StatSure device [26]; no data are available for the Oral-Eze device. Differences in collected OF volume could potentially explain the differences in THCCOOH and CBN concentrations. The low CBD prevalence and high THC variability immediately after smoking may contribute to the lack of significant differences for these analytes. Differences in cannabinoid recovery from the pad do not explain differences in concentrations. For the StatSure device, cannabinoid recoveries from the pad were 65.5-68.1%, 62.2-65.9%, 71.3-73.9%, and 65.1-71.2% for THC, CBD, CBN, and THCCOOH, respectively. For the Oral-Eze device, cannabinoid recoveries from the pad

were 42.5-48.8%, 33.5-47.7%, 35.6-58.7%, and 68.1-86.2% for THC, CBD, CBN, and THCCOOH, respectively [25]. The lower cannabinoid recoveries for THC, CBD, and CBN from the Oral-Eze pads are inconsistent with higher Oral-Eze concentrations. Other potential explanations for the differences in concentrations are placement of the device in the mouth (under the tongue for the StatSure and between the gum and the cheek for the Oral-Eze), differences in pad composition and thickness, differences in elution/stabilizing buffer, and sequential (rather than simultaneous) OF collections. Concentration differences due to analyte instability are not expected, as all but 5 Oral-Eze samples were analyzed within 24h of collection.

To our knowledge, no other study evaluated differences in authentic cannabinoid OF concentrations among collection devices after cannabis smoking. Langel *et al.* evaluated analyte recovery from fortified samples and collected OF volume from 8 OF devices; however, concentrations from authentic OF samples were not evaluated [26]. One study compared expectorated and StatSure OF samples and reported high concentration differences between the two collection methods [20]. In general expectorated THC concentrations were 6 times higher than StatSure concentrations, and consecutively collected expectorated samples varied much more in concentration than consecutively collected StatSure samples. The authors suggest that THC adsorption to cell debris in the OF could explain higher concentrations in expectorated OF, and that inhomogeneous THC distribution in the mouth could explain the higher variability in the expectorated OF compared to the StatSure (which is always placed under the tongue). Others documented no significant differences between simultaneously collected OF with the Quantisal [21] or Intercept devices [22].

Given the higher THCCOOH concentrations in the Oral-Eze samples, it is not surprising that THCCOOH detection rates are higher with this device (Figures 1 and 2). THC 1µg/L or THCCOOH 20ng/L provided the longest detection times in occasional and frequent smokers (Oral-Eze and StatSure). Due to low THCCOOH prevalence in occasional smokers, requiring a positive THCCOOH 20ng/L (e.g. THC 1µg/L and THCCOOH 20ng/L) greatly reduced detection rates in this cohort for the Oral-Eze and StatSure devices (Figure 1). We previously reported that higher THCCOOH OF concentrations in frequent smokers were due to their frequent past self-administered cannabis intake and high THC body burden [24].

No differences between t_{last} were observed between collection devices, except for THC 2µg/L. In this case, StatSure OF samples had a later t_{last} . It is unclear why there was a significant difference for this cutoff, as Oral-Eze concentrations were not significantly different. However, in one participant (participant K), THC concentrations were just above the 2µg/L cutoff with the StatSure device, but just below the 2µg/L cutoff with the Oral-Eze between 8-21h post smoking, possibly explaining the significantly later t_{last} for StatSure samples. Frequent smokers' t_{last} were generally later; had we captured the true t_{last} and not had to assign 30h assigned as 30h for statistical purposes, group differences could have differed.

When considering screening and THC confirmation tests, the DrugTest 5000 was generally the limiting factor in the positivity rate due to its higher 5µg/L cutoff compared to GCMS 1 and 2µg/ cutoffs (Figure 2). The only exception was in occasional smokers when THCCOOH 20ng/L was required for positive confirmation due to low THCCOOH OF prevalence in this group. These data are consistent with our previously reported evaluation of the DrugTest 5000 in chronic frequent cannabis smokers [19]. Similarly, detection times were reduced with the DrugTest 5000 (Table 2), and differences between occasional and frequent smokers' t_{last} were reduced except when THCCOOH was a required confirmation analyte.

Diagnostic sensitivity and efficiency following controlled smoked cannabis were slightly lower than previously reported; however, diagnostic specificity was higher [19]. This can be explained by the higher proportion of true negatives in this study compared to our previous study. Others reported diagnostic sensitivity, specificity, and efficiency of 53-93, 71-99 and 84-94.3%, respectively, (confirmation cutoffs of 1-10µg/L OF THC) [12-13,16-17]. Our data are consistent with our previously published data, indicating that the highest diagnostic efficiency was achieved with a 2µg/L THC confirmation cutoff. Occasional smokers had a lower sensitivity than frequent smokers (when considering only THC for confirmation) due to the generally higher concentrations in frequent smokers' OF at the later time points. This resulted in a lower incidence of true positives in occasional smokers. Similarly, diagnostic sensitivity, specificity, and efficiency improved in occasional and frequent smokers when considering samples collected up to 6 or 8h post dose, due to the higher incidence of true positive tests. This is consistent with two prior studies that documented higher sensitivities in samples collected recently after smoking [18,12].

Requiring THCCOOH (THC *and* THCCOOH statement) for confirmation generally reduced diagnostic specificity and efficiency, as it increased the number of DrugTest 5000 false positives, especially in frequent smokers. Due to the low LOQ for THCCOOH and high cannabinoid body burden in frequent smokers, THCCOOH remained positive when THC was no longer detected in OF, increasing the detection rate and window of detection [27].

Conclusion

We document for the first time OF cannabinoid disposition in occasional and chronic frequent cannabis smokers with an on-site screening device and with two OF collection devices following controlled smoked cannabis. We report significant differences in THCCOOH and cannabinol, but not THC concentrations between OF collection devices, which may influence OF data interpretation. The DrugTest 5000 on-site device has high diagnostic sensitivity, specificity, and efficiency for OF cannabinoid detection.

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Nonstandard abbreviations

OF	Oral fluid
THC	9-tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-THC
DUID	Driving under the influence of drugs
DRUID	Driving under the Influence of Drugs, Alcohol and Medicines
11-OH-THC	11-hydroxy-THC
CBD	cannabidiol
CBN	cannabinol
2D-GC-MS	two dimensional gas chromatography mass spectrometry
LOQ	Limit of quantification
TP	True positive
TN	True negative
FP	False positive
FN	False negative
SAMHSA	Substance Abuse and Mental Health Services Administration
t_{last}	time of last detection

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Fig 1.

Cannabinoid detection rates in Oral-Eze and StatSure oral fluid collection devices in occasional and frequent smokers following controlled smoking of a 6.8% 9-tetrahydrocannabinol (THC) cannabis cigarette

Fig 2.

Cannabinoid detection rates in DrugTest 5000 with Oral-Eze or StatSure oral fluid confirmation in occasional and frequent smokers following controlled smoking of a 6.8% 9-tetrahydrocannabinol (THC) cannabis cigarette

Table 1
Demographic characteristics and smoking histories for 14 frequent and 10 occasional cannabis smokers

Participant	Group	Race & Ethnicity	Gender	Age *	BMI *	Age 1st use *	Lifetime years smoked *	Time between last use and admission in h or d **	Number of days used in last 14 **	Average joint or joint equivalent/day or / month **
A	Frequent	AA	M	29.6	27.6	12	17.6	7.4 h	11	4/d
B	Frequent	AA	M	19.4	22.6	15	4.4	4.3 h	13	5/d
C	Frequent	AA	M	22.6	31.4	14	8.6	5.1 h	12	3/d
D	Frequent	W	M	25.5	23.0	13	12.5	3.9 h	14	20/d
E	Frequent	AA	F	19.9	32.4	11	8.9	2.6 h	14	3.5/d
F	Frequent	AA	M	24.2	27.4	13	11.2	23.2 h	12	1.5/d
G	Frequent	W	F	22.9	24.8	16	6.9	17.2 h	14	6/d
H	Frequent	AA	M	37.3	23.0	25	12.3	1.6 h	14	3/d
I	Frequent	AA	F	27.6	35.4	18	9.6	2.4 h	14	4/d
J	Frequent	AA	F	26.9	20.4	14	12.9	3.8 h	14	21/d
K	Frequent	AA	M	23.4	24.3	19	4.4	1.2 h	14	6/d
L	Frequent	AA	M	28.7	28.1	14	14.7	9.5 h	14	6/d
M	Frequent	AA	M	28.0	19.4	14	14.0	67.4 h [†]	2 [†]	2/m [‡]
N	Frequent	AA	M	23.8	30.7	14	9.8	273 h [†]	1 [†]	4/m [‡]
O	Occasional	W	M	25.6	29.4	16	9.6	16 d	0	2/m
P	Occasional	W	M	25.4	23.7	13	12.4	31 d	0	2/m
Q	Occasional	W	M	23.7	24.1	16	7.7	10 d	2	7/m
R	Occasional	AA	M	38.2	21.0	19	19.2	2 d	2	2/m
S	Occasional	M	M	41.3	22.0	16	25.3	7 d	5	10/m [‡]
T	Occasional	U	F	34.9	31.7	13	21.9	9 d	1	2/m
U	Occasional	AA	F	36.5	47.8	18	18.5	2 d	2	4/m
V	Occasional	M, H	M	22.5	25.2	13	9.5	86 d	0	6/m
W	Occasional	W	F	34.2	26.6	14	20.2	3 d	1	0.25/m
X	Occasional	AA	M	31.7	21.8	16	15.7	18 d	0	8/m
		Frequent	Mean	25.7	26.4	15.1	10.6	-	13.3	-

Participant	Group	Race & Ethnicity	Gender	Age [*]	BMI [*]	Age 1st use [*]	Lifetime years smoked [*]	Time between last use and admission in h or d ^{**}	Number of days ^{**} used in last 14 ^{**}	Average joint or joint equivalent/day or / month ^{**}
			StdDev	4.6	4.8	3.5	3.8	-	1.1	-
			Median	24.8	26.1	14.0	10.5	4.1 h	14.0	4.5/d
<hr/>										
	Occasional		Mean	31.4	27.3	15.4	16.0	-	-	-
			StdDev	6.7	8.0	2.1	6.0	-	-	-
			Median	33.0 [/]	24.7	16.0	17.1 [/]	9.5 [/]	1.0 [/]	3/m [/]

AA: African American, W: White, M: Mixed, U: Unknown, H: Hispanic or Latino

* Data collected at screening

Data collected prior to smoking

Self-reported data inconsistent with biological sample concentrations. Data excluded from mean and median

^t: Self-reported average use at screening of 0.5 joints, 3-4 times per monthSignificant difference between groups ($p < 0.05$)

Table 2

Median (range) time of last cannabinoid detection by screening and confirmation tests for 14 frequent and 10 occasional cannabis smokers following controlled smoking of 6.8% 9-tetrahydrocannabinol (THC) cigarette

	Occasional Smokers (h)	Frequent Smokers (h)	Mann Whitney (U)	Effect Size (r)	p
DrugTest 5000 Positive	12 (4 – 24)	21 (1 – 30 ^d)	43.5	–0.319	0.12
Oral-Eze THC 1 µg/L (DRUID)	21 (8 – 28)	28 (10.5 – 30)	29.5	–0.473	<0.05
Oral-Eze THC 2 µg/L (SAMHSA)	13.5 (5 – 26)	21 (6 – 30)	34	–0.406	0.051
Oral-Eze THCCOOH 20 ng/L	8 (1 – 21)	30	0	–0.923	<0.001
Oral-Eze THC 1 µg/L or THCCOOH 20 ng/L	21 (13.5 – 28)	30	0	–0.932	<0.001
Oral-Eze THC 1 µg/L and THCCOOH 20 ng/L	6.5 (1 – 13.5)	30	2.0	–0.822	<0.001
Oral-Eze THC 2 µg/L or THCCOOH 20 ng/L	13.5 (6 – 26)	30	0	–0.932	<0.001
Oral-Eze THC 2 µg/L and THCCOOH 20 ng/L	6.5 (1 – 13.5)	26 (6 – 30)	10.5	–0.711	<0.001
DrugTest 5000 Positive, Oral-Eze THC 1 µg/L	8.25 (2 – 21)	21 (1 – 30)	34	–0.404	0.053
DrugTest 5000 Positive, Oral-Eze THC 2 µg/L	7 (2 – 13.5)	12 (1 – 26)	38	–0.312	0.15
DrugTest 5000 Positive, Oral-Eze THC 1 µg/L or THCCOOH 20 ng/L	8.25 (2 – 21)	21 (1 – 30)	36.5	–0.371	0.078
DrugTest 5000 Positive, Oral-Eze THC 1 µg/L and THCCOOH 20 ng/L	4.5 (1 – 13.5)	21 (1 – 30)	19.0	–0.600	<0.01
DrugTest 5000 Positive, Oral-Eze THC 2 µg/L or THCCOOH 20 ng/L	7 (2 – 13.5)	21 (1 – 30)	26.0	–0.508	<0.05
DrugTest 5000 Positive, Oral-Eze THC 2 µg/L and THCCOOH 20 ng/L	4.5 (1 – 13.5)	12 (1 – 26)	27.0	–0.467	<0.05
StatSure THC 1 µg/L (DRUID)	21 (10.5 – 28)	28 (10.5 – 30)	27.5	–0.494	<0.05
StatSure THC 2 µg/L (SAMHSA)	12 (8 – 24)	24 (6 – 30)	27	–0.520	<0.01
StatSure THCCOOH 20 ng/L	4 (0 – 24)	30 (10.5 – 30)	5.0	–0.802	<0.001
StatSure THC 1 µg/L or THCCOOH 20 ng/L	21 (10.5 – 28)	30 (28 – 30)	2.0	–0.868	<0.001
StatSure THC 1 µg/L and THCCOOH 20 ng/L	4 (0 – 13.5)	25 (10.5 – 30)	9.0	–0.732	<0.001
StatSure THC 2 µg/L or THCCOOH 20 ng/L	13.5 (8 – 24)	30 (21 – 30)	5.0	–0.803	<0.001
StatSure THC 2 µg/L and THCCOOH 20 ng/L	4 (0 – 13.5)	24 (6 – 30)	10.5	–0.715	<0.001
DrugTest 5000 Positive, StatSure THC 1 µg/L	5.5 (2 – 21)	13.5 (1 – 30)	37.5	–0.359	0.09
DrugTest 5000 Positive, StatSure THC 2 µg/L	5.5 (2 – 21)	13.5 (1 – 30)	37.5	–0.359	0.09
DrugTest 5000 Positive, StatSure THC 1 µg/L or THCCOOH 20 ng/L	5.5 (2 – 21)	21 (1 – 30)	29.5	–0.462	<0.05
DrugTest 5000 Positive, StatSure THC 1 µg/L and THCCOOH 20 ng/L	2 (0 – 13.5)	13.5 (1 – 30)	22.0	–0.559	<0.01
DrugTest 5000 Positive, StatSure THC 2 µg/L or THCCOOH 20 ng/L	5.5 (2 – 21)	21 (1 – >30)	29.5	–0.462	<0.05
DrugTest 5000 Positive, StatSure THC 2 µg/L and THCCOOH 20 ng/L	2 (0 – 13.5)	13.5 (1 – 30)	22.0	–0.559	<0.01

Median (range) times of last detection between Oral-Eze and StatSure oral fluid collection devices following controlled smoked cannabis of a 6.8% tetrahydrocannabinol (THC) cigarette in 14 frequent and 10 occasional cannabis smokers

Table 3

Cutoff	Oral-Eze t_{last} (h)	StatSure t_{last} (h)	Z	p
THC 1 µg/L (DRUID)	24 (8 – 30)	26 (10.5 – 30)	-0.448	0.688
THC 2 µg/L (SAMHSA)	13.5 (5 – 30)	21 (6 – 30)	-1.980	<0.05
THCCOOH 20 ng/L	30 (1 – 30)	24 (0 – 30)	-1.260	0.221
THC 1 µg/L or THCCOOH 20 ng/L	30 (13.5 – 30)	28 (10.5 – 30)	0	1.0
THC 1 µg/L and THCCOOH 20 ng/L	21 (1 – 30)	13.5 (0 – 30)	-0.748	0.469
THC 2 µg/L or THCCOOH 20 ng/L	30 (6 – 30)	24 (8 – 30)	-0.597	0.594
THC 2 µg/L and THCCOOH 20 ng/L	13.5 (1 – 30)	13.5 (0 – 30)	-1.067	0.298
DrugTest 5000 Positive, THC 1 µg/L	13.5 (1 – 30)	10.5 (1 – 30)	-2.023	0.063
DrugTest 5000 Positive, THC 2 µg/L	9.25 (1 – 26)	10.5 (1 – 30)	0	1.0
DrugTest 5000 Positive, THC 1 µg/L or THCCOOH 20 ng/L	13.5 (1 – 30)	10.5 (1 – 30)	-0.267	0.832
DrugTest 5000 Positive, THC 1 µg/L and THCCOOH 20 ng/L	8 (1 – 30)	8 (0 – 30)	-0.682	0.514
DrugTest 5000 Positive, THC 2 µg/L or THCCOOH 20 ng/L	13.5 (1 – 30)	10.5 (1 – 30)	-1.381	0.178
DrugTest 5000 Positive, THC 2 µg/L and THCCOOH 20 ng/L	8 (1 – 26)	8 (0 – 30)	-0.039	0.988

Table 4

Performance characteristics for the Draeger DrugTest 5000 on-site test with a 5 µg/L 9-tetrahydrocannabinol (THC) screening cutoff in oral fluid with different confirmation cutoffs

		TP	TN	FP	FN	Total	Sensitivity	Specificity	Efficiency
Oral-Eze THC 1 µg/L (DRUID)	Overall	202	90	1	101	394	66.7	98.9	74.1
	Occasional	65	56	1	44	166	59.6	98.2	72.9
	Frequent	137	34	0	57	228	70.6	100	75.0
Oral-Eze THC 2 µg/L (SAMHSA)	Overall	195	128	8	63	394	75.6	94.1	82.0
	Occasional	64	74	2	26	166	71.1	97.4	83.1
	Frequent	131	54	6	37	228	78.0	90.0	81.1
Oral-Eze THC 1 µg/L or THCCOOH 20 ng/L	Overall	202	55	1	136	394	59.8	98.2	65.2
	Occasional	65	54	1	46	166	58.6	98.2	71.7
	Frequent	137	0	1	90	228	60.4	100	60.5
Oral-Eze THC 1 µg/L and THCCOOH 20 ng/L	Overall	167	125	36	66	394	71.7	77.6	74.1
	Occasional	31	90	35	10	166	75.6	72.0	72.9
	Frequent	136	35	1	56	228	70.8	97.2	75.0
Oral-Eze THC 2 µg/L or THCCOOH 20 ng/L	Overall	201	70	2	121	394	62.4	97.2	68.8
	Occasional	64	68	2	32	166	66.7	97.1	79.5
	Frequent	137	2	0	89	228	60.6	100	61.0
Oral-Eze THC 2 µg/L and THCCOOH 20 ng/L	Overall	161	148	42	43	394	78.9	77.9	78.4
	Occasional	31	94	35	6	166	83.8	72.9	75.3
	Frequent	130	54	7	37	228	77.8	88.5	80.7
Oral-Eze THC 2 µg/L (6h post-smoking)	Overall	160	25	0	27	212	85.6	100	87.3
	Occasional	54	20	0	14	88	79.4	100	84.1
	Frequent	106	5	0	13	124	89.1	100	89.5
Oral-Eze THC 2 µg/L (8h post-smoking)	Overall	173	28	1	33	235	84.0	96.6	85.5
	Occasional	58	22	0	18	98	76.3	100	81.6
	Frequent	115	6	1	15	137	88.5	85.7	88.3
StatSure THC 1 µg/L (DRUID)	Overall	196	94	7	98	395	66.7	93.1	73.4
	Occasional	64	58	2	42	166	60.4	96.7	73.5
	Frequent	132	36	5	56	229	70.2	87.8	73.3
StatSure THC 2 µg/L (SAMHSA)	Overall	191	125	12	67	395	74.0	91.2	80.0
	Occasional	64	73	2	27	166	70.3	97.3	82.5
	Frequent	127	52	10	40	229	76.0	83.8	78.2
StatSure THC 1 µg/L or THCCOOH 20 ng/L	Overall	200	66	3	126	395	61.3	95.7	67.3
	Occasional	64	57	2	43	166	59.8	96.6	81.9
	Frequent	136	9	1	83	229	62.1	90	63.3
StatSure THC 1 µg/L and THCCOOH 20 ng/L	Overall	140	144	63	48	395	74.5	69.6	71.9
	Occasional	11	97	55	3	166	78.6	63.8	65.1
	Frequent	129	47	8	45	229	74.1	85.5	76.8
StatSure THC 2 µg/L or THCCOOH 20 ng/L	Overall	200	86	3	106	395	65.4	96.6	72.4
	Occasional	64	72	2	28	166	69.6	97.3	81.9

			TP	TN	FP	FN	Total	Sensitivity	Specificity	Efficiency
StatSure THC 2 µg/L and THCCOOH 20 ng/L		Frequent	136	14	1	78	229	63.6	93.3	65.5
		Overall	135	155	68	37	395	78.5	69.5	73.4
		Occasional	11	97	55	3	166	78.6	63.8	65.1
		Frequent	124	58	13	34	229	78.5	81.7	79.5
StatSure THC 2 µg/L (6h post-smoking)		Overall	158	26	2	26	212	85.9	92.9	86.8
		Occasional	54	19	0	15	88	78.3	100	82.6
		Frequent	104	7	2	11	124	90.4	77.8	89.5
		Overall	172	28	2	33	235	83.9	93.3	85.1
StatSure THC 2 µg/L (8h post-smoking)		Occasional	58	20	0	20	98	74.4	100	79.6
		Frequent	114	8	2	13	137	89.8	80.0	89.1
		Overall	172	28	2	33	235	83.9	93.3	85.1
		Occasional	58	20	0	20	98	74.4	100	79.6

TN: True positive; TN: True negative; FP: False positive; FN: False negative; THC: 9-tetrahydrocannabinol; DRUID: Driving under the Influence of Drugs, Alcohol and Medicines; SAMHSA: Substance Abuse and Mental Health Services Administration; THCCOOH: 11-nor-9-carboxy-THC.