Cannabinoid Stability in Authentic Oral Fluid after Controlled Cannabis Smoking

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Abstract

BACKGROUND—Defining cannabinoid stability in authentic oral fluid (OF) is critically important for result interpretation. There are few published OF stability data, and of those available, all employed fortified synthetic OF solutions or elution buffers; none included authentic OF following controlled cannabis smoking.

METHODS—An expectorated OF pool and a pool of OF collected with Quantisal™ devices were prepared for each of 10 participants. Δ^9-Tetrahydrocannabinol (THC), 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), and cannabinol (CBN) stability in each of 10 authentic expectorated and Quantisal-collected OF pools were determined after storage at 4 °C for 1 and 4 weeks and at −20 °C for 4 and 24 weeks. Results within ±20% of baseline concentrations analyzed within 24 h of collection were considered stable.

RESULTS—All Quantisal OF cannabinoid concentrations were stable for 1 week at 4 °C. After 4 weeks at 4 °C, as well as 4 and 24 weeks at −20 °C, THC was stable in 90%, 80%, and 80% and THCCOOH in 89%, 40%, and 50% of Quantisal samples, respectively. Cannabinoids in expectorated OF were less stable than in Quantisal samples when refrigerated or frozen. After 4 weeks at 4 and −20 °C, CBD and CBN were stable in 33%–100% of Quantisal and expectorated samples; by 24 weeks at −20 °C, CBD and CBN were stable in 44%.

CONCLUSIONS—Cannabinoid OF stability varied by analyte, collection method, and storage duration and temperature, and across participants. OF collection with a device containing an elution/stabilization buffer, sample storage at 4 °C, and analysis within 4 weeks is preferred to maximize result accuracy.

Oral fluid (OF)[2] is a valuable alternative matrix for drug testing because it provides rapid, noninvasive, and directly observable sample collection (1), offering advantages for drug

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testing in settings of pain management, the workplace, and possible driving under the influence (2). Controlled drug administration studies define the spectrum and time course of analyte concentrations and windows of drug detection (3-4). Understanding cannabinoid OF pharmacokinetics is particularly critical because cannabis has the highest prevalence in many drug testing programs (5).

Drug stability is an important consideration in the interpretation of drug concentrations for clinical and forensic purposes. Δ^9-Tetrahydrocannabinol (THC), the primary psychoactive cannabis constituent and predominant OF analyte after smoking, degrades when exposed to air (6), acid (7), increased temperature, and light (8-9) and also adsorbs readily to glass, plastic, and precipitant material (10). There are few data on cannabinoid stability in OF. A <20% THC loss in fortified synthetic OF collected with the Quantisal™ device and stored at 2–8 °C for 14 days has been observed (11). THC, cannabidiol (CBD), cannabinol (CBN), and Δ^9-tetrahydrocannabinolic acid are stable in Quantisal samples stored at 4 °C for 10 days in fortified synthetic OF; cannabinoids, other than CBN, decrease approximately 50% at room temperature over the same period (12). With the Intercept® OF collection device, 13%, 45%, and 39% THC loss in fortified OF has been reported after 2 weeks at –20, 4, and 21 °C, respectively; after 6 weeks, 21%, 87%, and 86% THC losses occur (13). Cannabinoid stability in fortified synthetic OF could differ significantly from fortified authentic OF samples and also from authentic OF collected after cannabis smoking.

Although the proposed Substance Abuse and Mental Health Services Administration workplace drug-testing guidelines and the Driving under the Influence of Drugs, Alcohol and Medicines guidelines suggest monitoring only for THC in OF (14-15), we and others documented the importance of quantifying 11-nor-9-carboxy-THC (THCCOOH), CBD, and CBN (12, 16-19). THCCOOH was not present in cannabis smoke (20) and not detected in OF of individuals subjected to 3 h of extensive passive smoke exposure (21). THCCOOH increases in OF during round-the-clock oral THC exposure, whereas THC concentrations from previously self-administered smoked cannabis decrease to undetectable concentrations (22). In chronic daily cannabis smokers during prolonged abstinence (16), analysis of THCCOOH, CBD, and CBN, in addition to THC, can establish recent cannabis intake and differentiate new use from residual THC excretion, especially important for driving under the influence of drugs and other accident investigations. Thus, knowledge of stability of multiple cannabinoids in authentic OF is needed for valid interpretation.

In this study, we evaluated THC, THCCOOH, CBD, and CBN stability in authentic OF samples during refrigerated and frozen storage after controlled smoked cannabis administration. OF from 10 different individuals allowed assessment of intersubject stability differences.

Materials and Methods

PARTICIPANTS

Inclusion criteria were age 18–45 years, self-reported cannabis smoking at least twice per month during the 3 months before study entry, blood pressure 140 (systolic) and 90 (diastolic) mmHg, heart rate 100 bpm, and electrocardiogram without clinically relevant abnormalities. A positive urine cannabinoid test also was required as determined by the iScreen™ One Step Drug Card (Instant Technologies), a lateral flow chromatographic immunoassay with a 50-μg/L THCCOOH cutoff. Exclusion criteria were history or presence of clinically significant illness or adverse event associated with cannabis intoxication, 450

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[2]Nonstandard abbreviations: OF, oral fluid; THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; CBN, cannabinol; THCCOOH, 11-nor-9-carboxy THC; 11-OH-THC, 11-hydroxy-THC; LOQ, Limit of quantification.

mL blood donation within the previous 30 days, interest or participation in drug abuse treatment within 60 days, and pregnant or nursing females. The study was approved by the National Institute on Drug Abuse Institutional Review Board, and participants gave voluntary written informed consent.

OF STABILITY SAMPLE COLLECTION AND ANALYSIS

Participants resided on a closed research unit the night before drug administration. OF was collected with the Quantisal device (Immunalysis) and by expectoration at −0.5, 0.25, 0.5, 1, 2, 3, 4, 6, and 22 h post dose with respect to the time when ad libitum cannabis smoking of a 6.8% THC cigarette was initiated (maximum smoking time 10 min). The Quantisal collection pad collected 1.0 ± 0.1 mL OF. Subsequently, the pad was placed into 3 mL elution/stabilizing buffer for 19–24 h to elute drug analytes, yielding a 1:4 OF dilution. The OF–buffer mixture was decanted into Nunc Cryo-Tubes™ (Thermo Scientific). Participants also expectorated into polypropylene tubes until a minimum of 3 mL OF was collected, or for 5 min, whichever occurred first. OF was centrifuged and decanted into a Nunc CryoTube. OF samples were primarily employed for pharmacokinetic analyses (17); however, small portions at each time point through 6 h were combined for each participant, creating a Quantisal and an expectorated pool from each individual. We also included 22-h OF collections in the expectorated pool because of limited OF volume. After thorough vortex mixing, each pool was aliquoted into 5 Nunc CryoTubes for stability determinations. One aliquot was analyzed within 24 h for baseline concentration, 2 aliquots were stored at 4 °C and analyzed after 1 and 4 weeks, and 2 aliquots were stored at −20 °C and analyzed after 4 and 24 ± 2 weeks.

OF THC, CBD, CBN, 11-hydroxy-THC (11-OH-THC), and THCCOOH were quantified by use of a previously published 2-dimensional GC-MS method for Quantisal samples (23), and with minor sample preparation modifications in expectorated OF (24). Limits of quantification (LOQ) in Quantisal samples were 0.5 μg/L for THC, CBD, and 11-OH-THC; 1 μg/L for CBN; and 7.5 ng/L for THCCOOH and in expectorated samples were 0.25 μg/L for THC, CBD, and 11-OH-THC; 1 μg/L for CBN; and 5 ng/L for THCCOOH.

DATA ANALYSIS

IBM SPSS Statistics version 19.0 and Microsoft Excel were employed for statistical evaluation. Cannabinoid concentrations analyzed within 24 h served as baseline or 100% concentrations. Subsequent concentration changes are presented as %baseline, determined as [(stored sample concentration/baseline concentration) × 100]. Concentration changes <20% were considered stable. If %baseline could not be determined because of baseline concentrations   LOQ, low OF volume requiring sample dilution yielding concentrations <LOQ, or chromatographic interferences, results were excluded from calculations. Nonparametric Spearman's test was employed for correlation analysis. Results with 2-tailed $P < 0.05$ were considered significant.

Results

THC, CBD, CBN, and THCCOOH concentrations were quantified in 10 Quantisal and 10 expectorated OF samples analyzed within 24 h or stored for 1 or 4 weeks at 4 °C, or 4 or 24 weeks at −20 °C (Figs. 1 and 2). 11-OH-THC was not detected in any samples at LOQ. Of 40 data points per analyte, %baseline concentration calculations could not be determined for 4 THCCOOH, 7 CBD, and 3 CBN Quantisal samples and for 7 THCCOOH, 10 CBD, and 16 CBN expectorated samples because of low baseline concentrations, insufficient sample volume, or chromatographic interferences. Descriptive statistical data for every valid sample are displayed in Table 1 and Fig. 3. However, median data encompassing all samples do not

describe the intersubject variability observed. Therefore, we also provide median and range data for stable and unstable samples separately for each storage condition in subsequent text. Individual data are needed to elucidate the range of stabilities possible for a single sample. Percentages of stable samples for each cannabinoid under the 4 storage conditions are shown in Fig. 4.

THC STABILITY

In OF collected with the Quantisal device, THC concentrations were stable in 10 participants' samples for 1 week and in 9 samples for 4 weeks at 4 °C, with median %baseline concentrations of 94.1% (range 82.3%–111.8%) (Table 1) and 95.3% (82.1%– 109.7%), respectively. The THC concentration in 1 sample at 4 weeks decreased to 77.9% of baseline. After 4 and 24 weeks at −20 °C, 8 participants' THC concentrations were stable, with median %baseline concentrations of 90.4% (84.0%–110.6%) and 90.9% (86.9%– 98.3%), respectively; THC concentrations in 2 participants' samples decreased to 54.4% and 17.1% after 4 weeks and 52.7% and 3.0%, respectively, after 24 weeks of −20 °C storage.

In expectorated OF, THC concentrations in 6 participants' samples after 1 week and 3 after 4 weeks at 4 °C were stable, with median %baseline concentrations of 92.6% (81.8%– 100.5%) and 83.8% (83.7%–102.7%), respectively. In the other 4 and 7 participants' samples, THC decreased to medians of 34.3% (9.2%–79.2%) and 30.9% (2.4%–76.2%), respectively. After 4 weeks at −20 °C, 4 participants' THC concentrations were stable with a median %baseline concentration of 86.5% (81.3%–119.2%), whereas 6 participants' THC samples decreased to a median of 49.0% (13.0%–73.9%). After 24 weeks at −20 °C, THC concentrations in all 10 participants' OF samples decreased to a median of 51.2% (6.8%– 67.9%) (Table 1). For participant I, THC concentrations in Quantisal and expectorated OF samples were higher than the upper LOQ when analyzed within 24 h of collection; samples were diluted and reanalyzed 72 h after collection. Inclusion of the THC data for participant I did not change median %baseline concentrations by more than ±9.1%.

THCCOOH STABILITY

In OF collected with the Quantisal device, THCCOOH concentrations were stable in 10 participants' samples after 1 week and in 8 of 9 samples after 4 weeks at 4 °C with median %baseline concentrations of 94.7% (range 81.1%–103.1%) (Table 1) and 95.5% (83.6%– 102.9%), respectively. THCCOOH concentration in 1 sample after 4 weeks was reduced to 64.5%. After 4 and 24 weeks at −20 °C, 4 of 10 and 5 of 7 participants' THCCOOH concentrations were stable, with medians of 91.0% (83.1%–96.9%) and 93.7% (81.3%– 99.3%), respectively; 6 and 2 participants' THCCOOH after 4 and 24 weeks decreased to medians of 71.9% (40.4%–78.9%) and 69.9% (for both), respectively.

In expectorated OF, 6 of 9 participants' THC-COOH concentrations after 1 week at 4 °C were stable, with a median %baseline concentration of 96.6% (88.9–109.2); 2 participants' OF concentrations decreased to 73.9% and 78.3%, 1 increased to 127.9%. After 4 weeks at 4 °C, 4 of 8 participants' THCCOOH concentrations were stable, with a median %baseline concentration of 97.0% (85.7%–110.4%); 2 participants' OF concentrations decreased to 20.7% and 73.1%, and 2 increased to 124.4% and 147.6%. After 4 weeks at −20 °C, 4 of 8 participants' THCCOOH OF concentrations were stable, with a median of 87.4% (80.4%– 103.1%); 4 decreased to a median of 68.3% (55.6%–73.1%). After 24 weeks at −20 °C, only 1 of 8 participant's THCCOOH OF concentration was stable (82.4%); 7 decreased to a median of 62.6% (54.3%–71.8%).

CBD STABILITY

In OF collected with the Quantisal device, all 9 participants' CBD concentrations after 1 and 4 weeks at 4 °C were stable, with median %baseline concentrations of 95.3% (range 82.1%–102.6%) and 91.8% (86.2%–101.5%), respectively (Table 1). After 4 weeks at −20 °C, 5 of 8 participants' CBD OF concentrations were stable (87.0%, 81.9%–106.0%); 3 decreased to a median of 75.9% (13.7%–78.9%). After 24 weeks at −20 °C, 5 of 7 participants' CBD concentrations were stable (88.4%, 81.2%–108.2%); 2 decreased to 75.2% and 76.6%.

In expectorated OF, 5 of 7 participants' CBD concentrations after 1 week at 4 °C were stable, with a median %baseline concentration of 98.4% (90.9%–115.6%); 2 decreased to 19.4% and 62.0%. After 4 weeks at 4 °C, 3 of 6 participants' CBD concentrations were stable, with a median %baseline of 89.9% (85.3%–93.8%); 3 decreased to a median of 72.7% (25.0%–72.7%). After 4 weeks at −20 °C, 3 of 9 participants' CBD concentrations were stable, with a median %baseline concentration of 86.1% (81.5%–91.5%); 6 decreased to a median of 54.7% (20.3%–66.7%). After 24 weeks at −20 °C, 1 of 8 participant's CBD concentration was stable at 115.0% of baseline; 7 decreased to a median of 50.0% (35.0%–69.4%).

CBN STABILITY

In OF collected with the Quantisal device, CBN concentrations in all 10 participants after 1 week at 4 °C were stable, with a median %baseline concentration of 100.0% (range 90.5%–117.6%) (Table 1). After 4 weeks at 4 °C, 6 of 8 participants' CBN concentrations were stable, with a median of 96.5% (85.3%–102.4%) of baseline; 2 increased to 130.6% and 135.1%. After 4 weeks at −20 °C, 7 of 9 participants' CBN OF concentrations were stable, with a median of 92.6% (81.7%–101.4%) of baseline; 2 increased to 127.5% and 142.3%. After 24 weeks at −20 °C, 3 of 10 participants' CBN concentrations were stable, with a median %baseline concentration of 100.8% (85.0%–117.6%); 6 increased to a median of 155.3% (130.9%–183.6%), and 1 decreased to 76.7%.

In expectorated OF, CBN OF concentrations in 6 of 6 participants after 1 week at 4 °C were stable, with a median %baseline of 91.0% (82.9%–116.7%) (Table 1). After 4 weeks at 4 °C, 4 of 5 participants' CBN OF concentrations were stable, with a median %baseline concentration of 89.9% (87.3%–118.6%); 1 decreased to 78.2%. After 4 weeks at −20 °C, 4 of 7 participants' CBN concentrations were stable, with a median of 94.1% (83.3%–104.4%) of baseline; 3 increased to a median of 132.1% (125.6%–132.6%). After 24 weeks at −20 °C, 3 of 6 participants' CBN concentrations were stable, with a median %baseline concentration of 109.0% (89.4%–119.0%); 2 increased to 192.1% and 245.0%; 1 decreased to 73.1%. CBN concentrations generally increased in the Quantisal and expectorated samples after 4 and 24 weeks of storage.

RELATIVE STABILITY AMONG CANNABINOIDS

Changes in Quantisal and expectorated OF THC concentrations were significantly correlated with changes in THCCOOH (n = 36; ρ = 0.387; P = 0.020 and n = 33; ρ = 0.378; P = 0.030, respectively) and CBD concentrations (n = 33; ρ = 0.460; P = 0.007 and n = 30; ρ = 0.754; P < 0.001, respectively) but not with changes in CBN concentrations (n = 37; P > 0.05 and n = 24; P > 0.05, respectively) when all stability samples under 4 storage conditions were combined.

RELATIVE STABILITY BETWEEN COLLECTION METHODS

For THC, THCCOOH, and CBD stability in Quantisal OF samples, storage temperature was a primary factor, whereas for CBN, storage duration was key. In expectorated OF samples, storage duration was more important than temperature for stability of all analytes. There

were fewer differences in stability between Quantisal and expectorated OF samples for CBN than for any other analyte. THC was the most stable analyte in Quantisal OF samples, whereas THC, THCCOOH, and CBD had similar poor stability in expectorated OF samples. These results suggest that the Quantisal buffer optimally stabilizes THC, the only analyte currently recommended for OF monitoring by Substance Abuse and Mental Health Services Administration, Driving under the Influence of Drugs, Alcohol and Medicines, and multiple jurisdictions, at 4 °C, with its stabilizing capacity decreased at −20 °C.

Discussion

This study is the first of which we are aware to evaluate cannabinoid stability in authentic OF samples after controlled cannabis smoking. Two strengths of the study design were analysis of samples within 24 h of collection after controlled cannabis smoking, allowing accurate determination of baseline OF concentrations, and preparation of individual pools for each of 10 different participants, enabling evaluation of intersubject variability. A third strength of the study was inclusion of authentic OF samples with a wide variety of THC concentrations. Even for the OF samples with the lowest THC baseline concentrations (17.5 and 6.4 μg/L for Quantisal and expectorated samples, respectively), decreases of >90% of baseline remained THC-positive at the specific cutoff concentrations. This is an important consideration for reanalysis, which may be required in forensic situations.

Overall, cannabinoids in OF collected with the Quantisal device were more stable and had more consistent quantifications than cannabinoids in expectorated OF. The stabilizing buffer in Quantisal-collected samples could have contributed to improved stability compared with expectorated OF. The buffer (mean pH 7.0 ± 0.1) may have prevented cannabinoid degradation by (*a*) stabilizing the pH of the OF, (*b*) retaining analytes in solution and thereby reducing adsorption to collection tube surfaces and/or precipitants, and/or (*c*) inhibiting enzymatic degradation. The high viscosity of expectorated OF also makes accurate pipetting more difficult, increasing cannabinoid quantification variability (24). Additionally, expectorated OF contains more miscellaneous mouth debris that may absorb cannabinoids. Mucous in expectorated OF may reduce cannabinoid binding to the sorbent bed of solid phase extraction columns, reducing analyte recovery during sample preparation. In addition, the Quantisal buffer dilutes OF, increasing volume available for analysis, and reducing viscosity improves precision in cannabinoid quantification. On the other hand, the device's volume indicator may fail immediately after smoking because of insufficient OF, preventing accurate measuring of sample volume; this would not be a problem if only qualitative results are required because such samples still produce positive results (17). However, OF should be re-collected for accurate quantitative results.

A limitation of the present study was the necessity for dilution of samples because of low OF sample volume, which sometimes yielded nonquantifiable results. Cannabis smoking produces "dry mouth" or xerostomia (25). Dilution increases the LOQ, reducing the number of samples that could be evaluated. Stabilities in these samples were excluded from median and range calculations, sometimes providing a misleading impression of stability. For instance, the %baseline range for Quantisal THC-COOH at −20 °C after 4 weeks was 40.4%–96.9%, whereas after 24 weeks it was 69.9%–99.3% (Table 1). This is because 3 samples had concentrations <LOQ after dilution. In such samples, individual losses of at least 32% and 85% of baseline for Quantisal THCCOOH and CBD and 21%, 58%, and 41% of baseline for expectorated THCCOOH, CBD, and CBN, respectively, were observed according to the adjusted LOQ. Therefore, it is important to evaluate individual stability results before forming conclusions on overall stability under specific storage conditions. However, this limitation was primarily associated with expectorated samples that consistently demonstrated poorer stability than Quantisal-collected samples. Furthermore, it

is important to determine stability in authentic OF rather than synthetic OF or stabilizing buffer in which limited volume would not be an issue.

Stability was defined as a concentration change less than ±20% of baseline to account for analytical imprecision (26). Apart from analytical imprecision, decreases in OF cannabinoid concentrations over time could be caused by irreversible cannabinoid binding to surfaces and/or precipitants (27), and/or by enzymatic degradation (28); >97% of OF is water, but electrolytes, immunoglobulins, enzymes, and other proteins are present (29-30). Many hormones and enzymes present in plasma were detected in OF, albeit in lower concentrations (31). Enzymes involved in cannabinoid metabolism also were expressed in human oral tissue cells (32-34). Cannabinoid chemical structure and free cannabinoid concentrations may decrease owing to the presence of these compounds. Additionally, OF flow rate, pH, and composition of electrolytes, proteins, enzymes, and other compounds (35) may differ between individuals; the microenvironment surrounding analytes is consequently different in each OF sample. These data demonstrate that cannabinoid stability varies by analyte, collection method, storage duration, and temperature, and across participants.

Degradation of THC to CBN, leading to CBN increases over time in stored cannabis plant material, has been documented in multiple studies (9, 36-37). Conversion was not directly to CBN but through various intermediates (8, 38). Cannabinoids other than THC also may be converted to CBN (39). Thus, it was not surprising that THC decreases did not significantly correlate with increases in CBN in the present study. These different stability characteristics should be carefully considered when multiple cannabinoids are included in drug-testing regulations.

THCCOOH may play a significant role in OF drug testing by excluding passive cannabis exposure (21), extending the cannabinoid detection window (16), and identifying THC intake by the oral route (22). We previously showed that during round-the-clock oral THC exposure, OF THC concentrations decreased over time, whereas THCCOOH concentrations increased (22). THCCOOH was a good marker of cannabinoid exposure because this metabolite was measureable in OF within 0.25 h after cannabis smoking (17). THCCOOH was stable in the majority of Quantisal samples for 4 weeks at 4 °C, but concentrations decreased over time at −20 °C. Expectorated samples showed poor THCCOOH stability even after 1 week. THCCOOH %baseline concentration increases up to 48% in 3 expectorated samples were possibly due to hydrolysis of conjugated THCCOOH. Moore et al. (40) documented free THCCOOH increases of 50%, 8%, and 65% after glucuronidase, sulfatase, and base hydrolysis treatments, respectively, in Quantisal OF samples.

Overall, cannabinoids in OF collected with the Quantisal device were generally stable for 4 weeks at 4 °C. Greater instability at −20 °C could be in part due to poorer stabilizing ability of the Quantisal buffer at freezing temperatures. Even with reduced stability after 24 weeks at − 20 °C, THC, THCCOOH, and CBD were stable in more than 44% of participants' Quantisal OF samples; in expectorated OF samples, 13% were stable. Thus, for cannabinoid OF testing, the Quantisal collection device is preferred over expectoration for sampling. We also suggest performing analyses within 4 weeks of storage at 4 °C, at least until further research documents stability beyond this time. Also, because each collection device contains proprietary stabilizing buffers, cannabinoid stability in OF must be determined for each type of collection device, limiting generalization of results to other collection devices, and strongly supporting the need for additional stability studies.

These data emphasize the importance of OF collection device design. Early in OF testing development, differences in the spectrum of analytes and concentrations were found between OF and urine, focusing recovery from the device on THC, and necessitating

development of cannabinoid immunoassays targeted for THC rather than THCCOOH. Our data suggest that the ability of the stabilizing/elution buffer to maintain cannabinoid integrity during storage is another key variable in obtaining accurate cannabinoid OF results.

Acknowledgments

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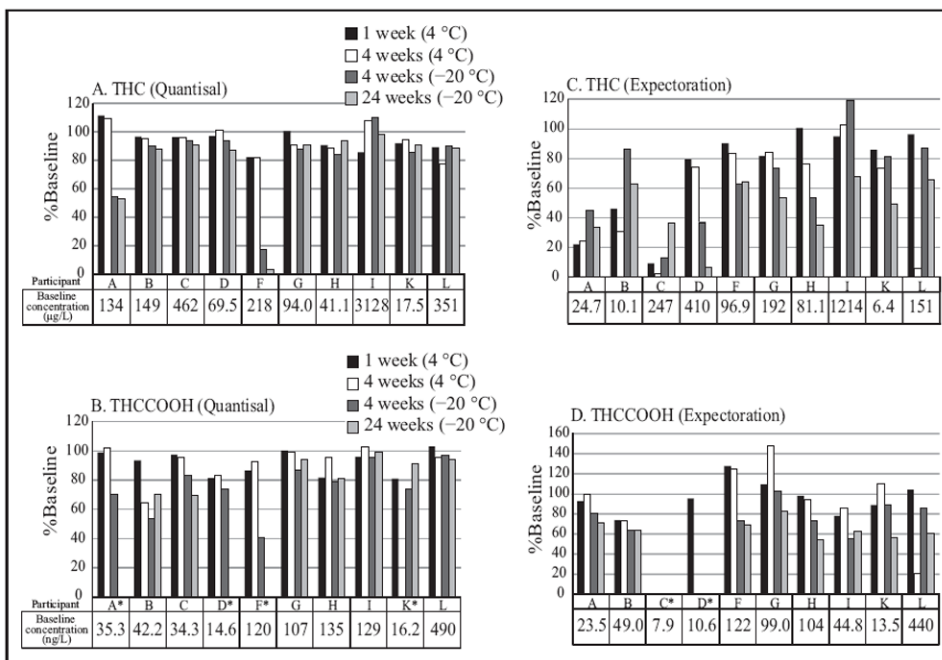
Fig. 1. THC and THCCOOH OF concentrations as %baseline concentration for each participant pool (n = 10)

OF was collected with the Quantisal device (A, B) and by expectoration (C, D) after storage for 1 week at 4 °C, 4 weeks at 4 °C, 4 weeks at −20 °C, and 24 weeks at −20 °C. *, missing data due to low sample volume or low baseline concentration.

Fig. 2. CBD and CBN OF concentrations as %baseline concentrations for each participant pool (n = 10)
OF was collected with the Quantisal device (A, B) and by expectoration (C, D) after storage for 1 week at 4 °C, 4 weeks at 4 °C, 4 weeks at −20 °C, and 24 weeks at −20 °C. *, missing data due to low sample volume, low baseline concentration, or chromatographic interference.
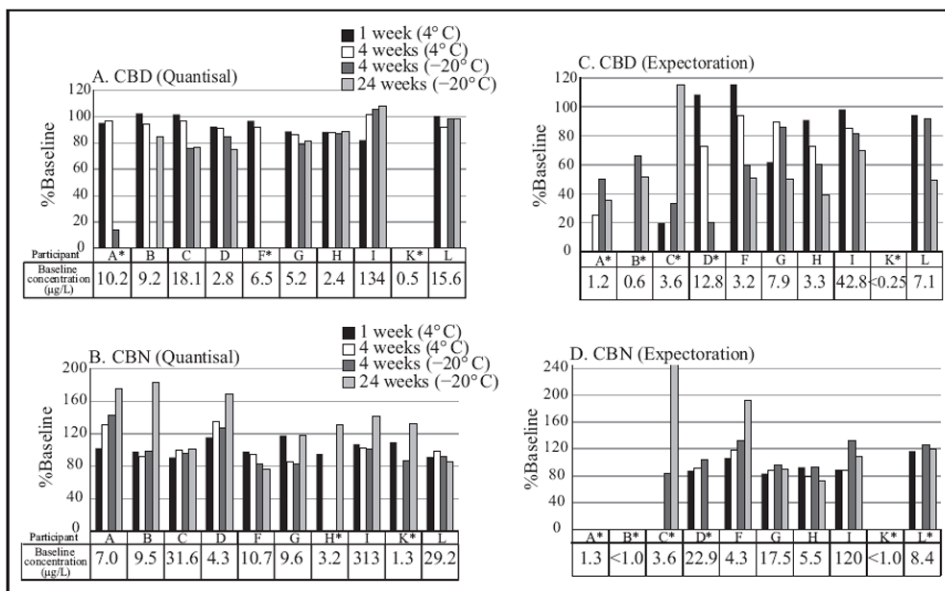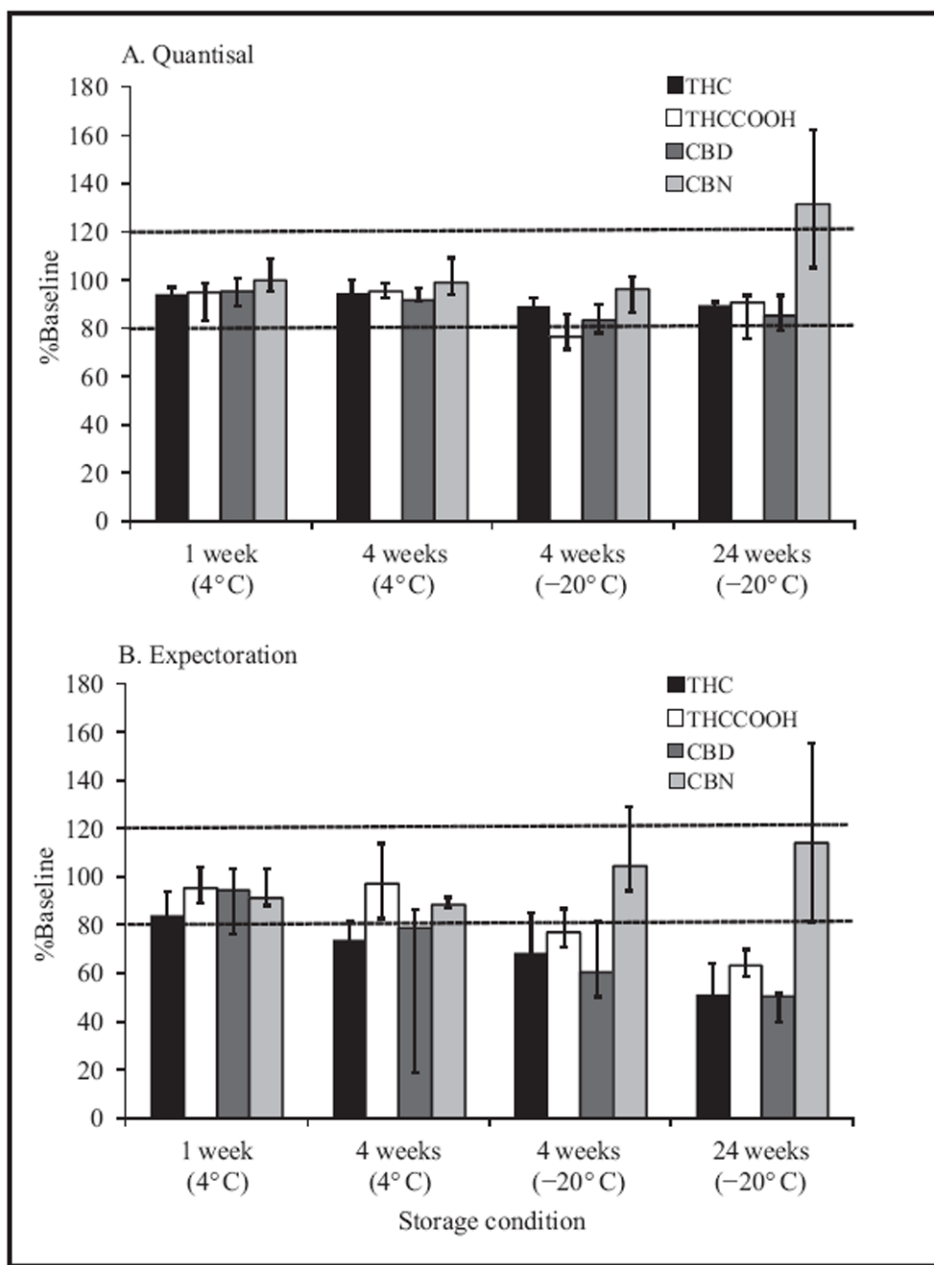
Fig. 3. Median cannabinoid concentrations in %baseline concentrations
Data counts vary depending on analyte and storage conditions (refer to Table 1). Error bars indicate interquartile ranges.
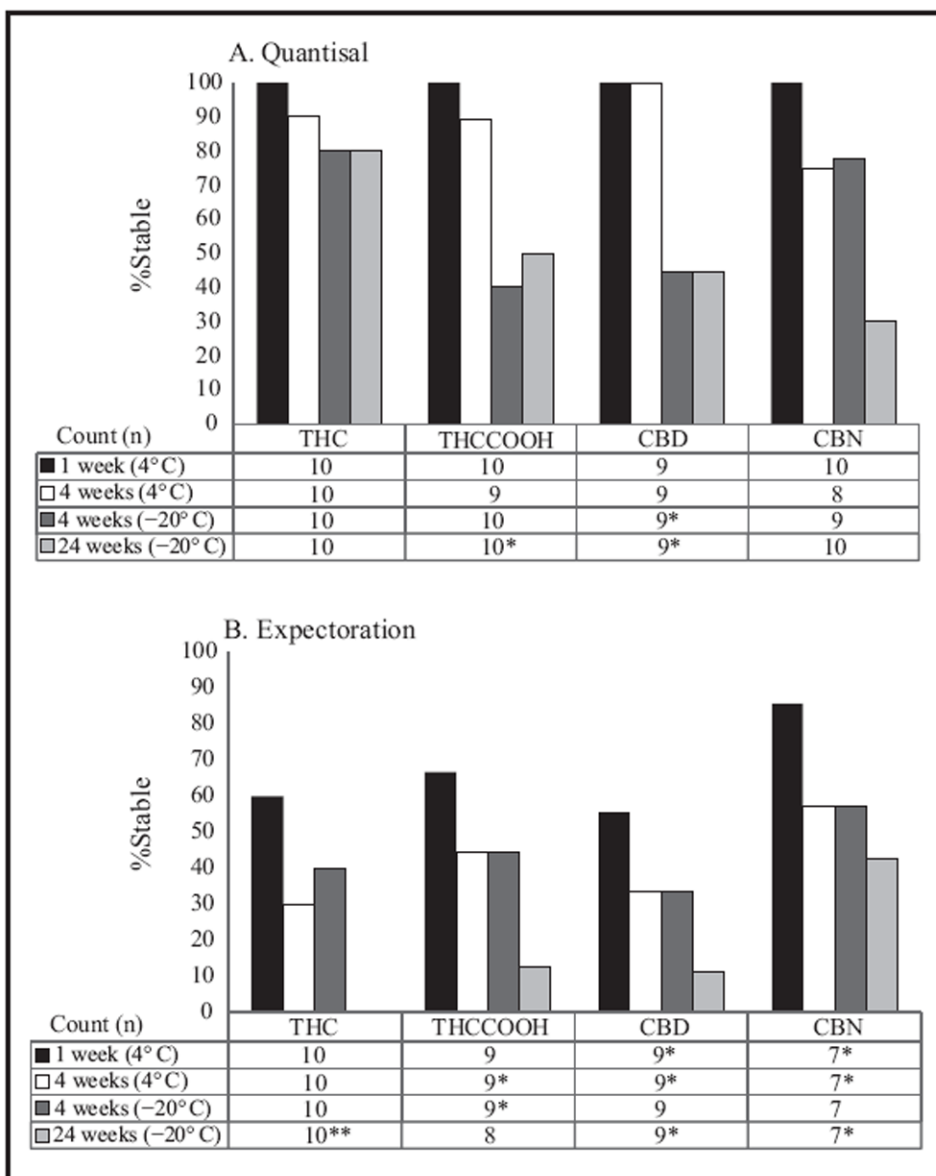
A. Quantisal

Count (n)	THC	THCCOOH	CBD	CBN
■ 1 week (4°C)	10	10	9	10
□ 4 weeks (4°C)	10	9	9	8
▦ 4 weeks (−20°C)	10	10	9*	9
▨ 24 weeks (−20°C)	10	10*	9*	10

B. Expectoration

Count (n)	THC	THCCOOH	CBD	CBN
■ 1 week (4°C)	10	9	9*	7*
□ 4 weeks (4°C)	10	9*	9*	7*
▦ 4 weeks (−20°C)	10	9*	9	7
▨ 24 weeks (−20°C)	10**	8	9*	7*

Fig. 4. Percentage of participants with stable %baseline cannabinoid concentrations (within 80%–120% of baseline) for 4 storage conditions
Data table includes total numbers of participants whose analyte stability could be determined. *, In 3 THCCOOH and 3 CBD Quantisal samples and 2 THCCOOH, 6 CBD, and 4 CBN expectorated samples analysis was required on dilution because of low sample volume, raising the LOQ for the sample. Thus, we were unable to determine the exact %baseline concentration, but calculated as [(LOQ × dilution factor/baseline concentration) × 100], concentrations were definitively determined to be <80% of the baseline concentration and, therefore, unstable. **, 0%; no participants had stable %baseline concentrations.

Table 1

Cannabinoid concentration changes from baseline for oral fluid samples collected with the Quantisal device (A) and by expectoration (B) after being refrigerated (4 °C for 1 and 4 weeks) and frozen (−20 °C for 4 and 24 weeks) storage.

	Storage condition	Count[a]	%Baseline, median[b]	%Baseline, mean[b]	%Baseline, SD	%Baseline, range[b]
A. Quantisal collection device						
THC	1 week, 4 °C	10	94.1	94.2	8.3	82.3–111.8
	4 weeks, 4 °C	10	94.8	94.4	10.1	77.9–109.7
	4 weeks, −20 °C	10	89.2	80.8	26.3	17.1–110.6
	24 weeks, −20 °C	10	89.6	78.3	29.2	3.0–98.3
THCCOOH	1 week, 4 °C	10	94.7	92.0	8.5	81.1–103.1
	4 weeks, 4 °C	9	95.5	92.3	11.9	64.5–102.9
	4 weeks, −20 °C	10	76.3	75.2	17.6	40.4–96.9
	24 weeks, −20 °C	7	90.8	85.5	12.0	69.9–99.3
CBD	1 week, 4 °C	9	95.3	94.3	6.9	82.1–102.6
	4 weeks, 4 °C	9	91.8	93.1	4.6	86.2–101.5
	4 weeks, −20 °C	8	83.4	78.4	28.0	13.7–106.0
	24 weeks, −20 °C	7	85.2	87.6	12.0	75.2–108.2
CBN	1 week, 4 °C	10	100.0	102.4	9.6	90.5–117.6
	4 weeks, 4 °C	8	98.9	104.7	18.1	85.3–135.1
	4 weeks, −20 °C	9	96.1	101.0	20.7	81.7–142.3
	24 weeks, −20 °C	10	131.6	131.3	37.3	76.7–183.6
B. Expectoration						
THC	1 week, 4 °C	10	83.8	70.7	32.8	9.2–100.5
	4 weeks, 4 °C	10	73.9	55.7	36.3	2.4–102.7
	4 weeks, −20 °C	10	68.3	65.8	30.3	13.0–119.2
	24 weeks, −20 °C	10	51.2	47.4	19.5	6.8–67.9
THCCOOH	1 week, 4 °C	9	95.3	96.5	16.3	73.9–127.9
	4 weeks, 4 °C	8	97.0	94.5	37.7	20.7–147.6
	4 weeks, −20 °C	8	76.8	78.0	15.0	55.6–103.1
	24 weeks, −20 °C	8	63.0	65.0	9.2	54.3–82.4

	Storage condition	Count[a]	%Baseline, median[b]	%Baseline, mean[b]	%Baseline, SD	%Baseline, range[b]
CBD	1 week, 4 °C	7	94.4	84.2	33.2	19.4–115.6
	4 weeks, 4 °C	6	79.0	73.2	25.2	25.0–93.8
	4 weeks, −20 °C	9	60.6	61.1	23.9	20.3–91.5
	24 weeks, −20 °C	8	50.3	57.5	25.3	35.0–115.0
CBN	1 week, 4 °C	6	91.0	96.0	13.0	82.9–116.7
	4 weeks, 4 °C	5	88.6	92.8	15.3	78.2–118.6
	4 weeks, −20 °C	7	104.4	109.4	20.4	83.3–132.6
	24 weeks, −20 °C	6	114.0	137.9	66.6	73.1–245.0

[a] Number of valid samples for %baseline concentration calculations.

[b] %Baseline = (concentration after storage under 1 of 4 conditions/baseline concentration) × 100.