Pharmacology
Research Paper

OPEN

PAIN REPORTS

Long-term effectiveness and safety of medical cannabis administered through the metered-dose Syqe Inhaler

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Abstract

Introduction: Preliminary clinical studies on medical cannabis (MC) treatment using the Syqe Inhaler showed short-term effectiveness and safety at very low and precise doses of MC.

Objectives: Here, we retrospectively analyzed "real-life" long-term data collected in real time on the potential effectiveness and safety of MC administered with this device.

Methods: Patients were monitored by Syqe's patient support program. (−)-Δ^9-trans-Tetrahydrocannabinol (Δ^9-THC) served as a dosage marker for full-spectrum MC. Pain intensity was evaluated using a numeric pain scale (NPS) from baseline to 120 days after treatment initiation. The change in quality of life (QoL) from baseline was evaluated. Adverse events (AEs) were followed up continuously for 15 months.

Results: Of the 143 patients (mean age 62 ± 17 years; 54% males) included in the analysis, most (72%) were diagnosed with chronic neuropathic pain. The stable daily dose, after a mean 26 ± 10 days of titration was 1,500 ± 688 μg aerosolized Δ^9-THC. Significant pain reduction, ranging from 22.8% in the intent-to-treat population to 28.4% in the population that reported baseline pain intensity ≥8 points on the NPS ($P < 0.001$), was observed. Ninety-two percent of patients reported improved QoL. Adverse events were reported mostly during the titration phase (34% of patients) and declined to ≤4% at 3 to 15 months. Only 7% of patients reported psychoactive AEs (anxiety and restlessness).

Conclusions: Medical cannabis treatment with the Syqe Inhaler demonstrated overall long-term pain reduction, QoL improvement, and a superior AE profile compared with administration of MC by conventional routes. Additional follow-up in a larger population is warranted.

Keywords: Cannabis, Medical use, Metered dose, Chronic pain

1. Introduction

The use of medical cannabis (MC) for treating pain in various medical conditions is on the rise worldwide[9] despite the ongoing debate on whether the low-quality evidence on its effectiveness[10,16] justifies potential harms associated with its use.[14,16] Furthermore, recommended titration regimens and stable effective and safe MC doses are not readily available because of the diverse cultivar selection, which differs in their phytocannabinoid and terpenoid profile,[3] and the multiple administration routes, each with a different bioavailability.[6] Hence, transforming MC into an acceptable medical treatment is a constant challenge.[20]

To achieve a systemic effect, MC is usually delivered orally or sublingually or by inhalation (smoking/vaporization). Both administration routes are characterized by considerable variability in the concentration of (−)-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) in the plasma.[15,17,21] Inhalation of MC is preferred by many patients, possibly because of its fast onset of effects.[17]

The Syqe Inhaler 1.1 (Trade name: SyqeAir, Syqe Medical, Tel Aviv-Yafo, Israel) is a novel metered selective-dose MC inhaler that provides a possible solution for the variability of Δ^9-THC blood levels after inhalation. The inhaler is configured to use a vapor chip (VC) to deliver an aerosol containing 250 or 500 μg Δ^9-THC as an indicator for phytocannabinoids, terpenoids, and other molecules from the whole inflorescence that are aerosolized

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.painrpts.com).

PR9 7 (2022) e1011

http://dx.doi.org/10.1097/PR9.0000000000001011

concomitantly with Δ^9-THC. The inhaler heats the medical grade cannabis to a temperature below combustion and engages automatic thermal and airflow controls that ensure precise, accurate, and high-efficiency delivery of the produced MC aerosol to the patient's lungs, independent of the inhalation pattern of the individual patient. The inhalation process is similar to shallow breathing (ie, 3–15 L/min): after the user inhales for 1.8 seconds, the inhaler airway becomes blocked and the user experiences resistance. The airway then opens creating a chase-air pulse that flushes the aerosolized MC past anatomic dead space and deep into the lungs, resulting in enhanced bio-availability.[2] An airflow modulation–lung interface reflux serves as an indication for the patient that the inhalation was completed successfully. The entire inhalation process lasts 2 to 5 seconds. For example, the duration of a single inhalation of 500 μg Δ^9-THC is 2.8 seconds.

In a study that evaluated the pharmacokinetics of MC using an earlier version of the inhaler, the maximal concentration in plasma (C_{max}) of 1,000 μg of aerosolized Δ^9-THC ranged from 26 to 53 ng/mL.[12] This C_{max} range was much narrower than the reported C_{max} range after cigarette smoking of MC under controlled conditions (~50–250 ng/mL[17]). In a randomized, double-blind, placebo-controlled trial that evaluated reduction of chronic pain with 500 μg and 1000 μg of aerosolized Δ^9-THC among patients with noncancer pain, average pain was reduced by 1.95 and 2.95 points (on a scale of 0–10), respectively, for 150 minutes. Pharmacokinetics evaluation after administration of 500 μg and 1,000 μg of aerosolized Δ^9-THC through the inhaler showed low variability in plasma concentration among subjects. Δ^9-THC plasma levels after administration of the 1,000-μg dose were twice as high as those after administration of the 500-μg dose, indicating stable dosing. Adverse events (AEs) were mostly mild, reversible, and receded rapidly.[2] Here, we retrospectively analyzed "real-life" long-term data collected in real time on the potential effectiveness of low-dose MC delivered by the Syqe Inhaler in reducing pain and other symptoms and on the safety of this mode of MC delivery.

2. Methods

2.1. Study design and setting

Syqe Medical provides all patients who use its metered-dose inhaler a free patient support program (PSP). On joining this program, the patients provide their informed consent, which allows data collection by Syqe's PSP nurse team. The PSP also includes a call center that archives every AE report.

In this study, we retrospectively analyzed the data of all patients who were enrolled in the program between September 2019 and October 2020. Analysis of the collected data was approved by the Technion - Israel Institute of Technology's Ethics Committee (#125-2021).

2.2. Device

The Syqe Inhaler 1.1 (**Fig. 1**) consists of a cartridge containing 60 preloaded VCs each containing a precise predefined amount of raw ground cannabis, which is free of pesticides, heavy metals (<0.2 ppm lead, <0.02 ppm mercury, and <0.02 ppm cadmium), stalks, and foreign materials, such as insects and other vermin. Microbiological purity is regularly confirmed (total aerobic microbial count of <10 colony forming units [CFU]/g; total yeast and mold count of <10 CFU/g; and absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and bile-tolerant gram-negative bacteria). Each cartridge contains a radiofrequency identification (RFID) label. The device identifies the RFID label both electronically and by distinct mechanical points on the cartridge. Without this authentication, the device will not recognize the cartridge and will not operate. The device requires minimal training before use and automatically generates logs of the inhalation process.

Figure 1. Syqe metered-dose Inhaler. The Syqe Inhaler as used (left image) and its internal components (right image).

2.3. Medical cannabis treatment

Each patient's treatment regimen (ie, MC dose and the number of inhalations) was individualized using a titration plan provided by Syqe Medical's PSP. Four different titration plans were available. The titration plan was chosen according to the patient's MC use before treatment initiation (when relevant), age, and relevant comorbidities, if any. Each titration plan set a maximum dose limit for the MC regimen (Supplemental Figure 1, available at http://links.lww.com/PR9/A162).

Each VC was preloaded with 13.5 ± 0.9 mg processed granulated pharmaceutical grade cannabis flowers (Bedrocan, Veendam, The Netherlands). The inhaler is electronically configured to deliver an aerosol containing either 250- or 500-μg Δ^9-THC dose from each VC.

Dose titration for naive patients (who had never smoked or vaped MC) began with two 250-μg Δ^9-THC doses per day, and only 1 daily dose of 250 μg Δ^9-THC in patients aged 80 years and older. Experienced patients, who had been smoking or vaping MC before initiating treatment with the metered-dose inhaler, started with four 500-μg Δ^9-THC doses per day. Thereafter, patients could add small incremental doses in accordance with their titration plan, which was subject to the absence of AEs for 3 consecutive days, or to the presence of tolerable AEs, which were defined as AEs perceived by the patient as ones not preventing him or her from continuing the treatment (eg, dry mouth or mild cough). Dose titration was supported and monitored by a PSP nurse, who assisted the patient in reaching a stable treatment regimen with as few AEs as possible. The titration ended once the patient achieved satisfactory symptom relief without any intolerable AEs, which were defined as AEs perceived by the patient as ones that prevented him or her from continuing the treatment or defined by the PSP nurse as potentially harmful.

Patients were allowed to use rescue (SOS) doses on top of their scheduled daily dose for breakthrough pain episodes.

2.4. Outcome measures

Average weekly pain intensity was measured by a numerical pain scale (NPS) ranging from 0 (no pain) to 10 (worst imaginable pain intensity).

Adverse events were assessed by an open question: "Have you experienced any AE since the last follow-up?" At each follow-up visit, patients were also asked about changes, if any, to their medication regimens other than MC administered by the inhaler.

Before conducting the analysis of the study's data (October 2020), all patients who had reached the stable-dose phase were contacted by the PSP nurses and were requested to report any perceived change from baseline, in their quality of life (QoL), using a 5-point Likert scale ("much worse," "worse," "no change," "better," or "much better").

2.5. Data collection

Data were collected by Syqe's PSP nurses based on outcomes that were reported by the patients. A baseline meeting, during which the nurse instructed the patient on using the inhaler, was held in person at the patient's home. In this meeting, the nurse asked the patient about previous MC treatments, comorbidities, medication history, and concomitant medications. Women were asked whether they were pregnant or breastfeeding. Pain intensity and patient demographics (age and sex) were also recorded.

Inasmuch as possible, the same PSP nurse collected data on pain intensity and AEs by phone at predefined times: 7, 14, 21, 30, 60, 90, and 120 days after treatment initiation. Additional scheduled calls were made by the PSP team for safety monitoring at 180 and 360 days after treatment initiation. At each call, women were also asked whether they were pregnant or breastfeeding. Patients were instructed to call the PSP support service at any time of the day or night if needed.

2.6. Statistical analysis

The intention-to-treat (ITT) population included all patients who were treated with the inhaler and had data for any time point. The per-protocol (PP) population included all patients who had data for all time points. Categorical variables are presented as number and percentage. Distribution was assessed by the Shapiro–Wilk test of normality. Data with nonnormal distribution is presented as median and interquartile range (IQR), and normally distributed data are presented as mean ± SD. R software (V.1.1.463) with lme4[7] and tidyverse[23] packages was used to analyze changes in outcome measures by generalized linear mixed-effect regression models[13] for the ITT analyses. Repeated measures analysis of variance (ANOVA) was used for the PP analysis. Differences were considered significant if the *P* value was lower than 0.05.

3. Results

3.1. Patient characteristics

At the time of data analysis (October 2020), 215 patients were enrolled and initiated treatment with MC using the Syqe Inhaler. Of them, 143 patients (54% male) with a mean age of 62 ± 17 years completed the titration and were eligible for the ITT analysis. Most patients (n = 103, 72%) were prescribed MC treatment using the metered-dose inhaler because of chronic neuropathic pain. Other diagnoses included chronic musculoskeletal pain (n = 14, 10%), cancer pain (n = 9, 6%), chronic nociplastic pain (n = 4, 3%), chronic visceral pain (n = 2, 1%), and medical conditions with concomitant chronic pain (n = 6, 4%). Five additional patients (3%) received MC with the inhaler for diagnoses other than chronic pain (symptomatic cancer, essential tremor, Parkinson disease, obsessive compulsive disorder, and multiple sclerosis). All etiologies for the indications are presented in Supplemental Table 1 (available at http://links.lww.com/PR9/A162). None of the female patients were pregnant or breastfeeding at enrollment.

Thirty-eight patients (27%) had comorbidities as follows: 23 (16%) had hypertension; 12 (8%) had diabetes; 3 (2%) had congestive heart failure; and 1 patient each had Meniere disease, emphysema, asthma, or Addison disease.

During the follow-up period, 28 patients (19.5%) dropped out from the PSP for the following reasons: 3 patients (2%) died because of causes unrelated to the treatment (2 from cancer and 1 patient with myasthenia gravis complicated by severe pneumonia), 7 patients (5%) withdrew because of financial reasons, 3 patients (2%) no longer needed MC treatment for their chronic pain (2 were cured by surgical interventions and a third patient was cured spontaneously), 8 patients (6%) reported worsening of their medical condition (eg, severe cardiac insufficiency, brain metastases, and severe muscle dystrophy affecting respiratory function), 5 patients (3%; all men) stopped treatment because of ineffectiveness, 1 patient (<1%) withdrew because of AEs, and 1 patient (<1%) because of regulatory reasons (**Fig. 2**).

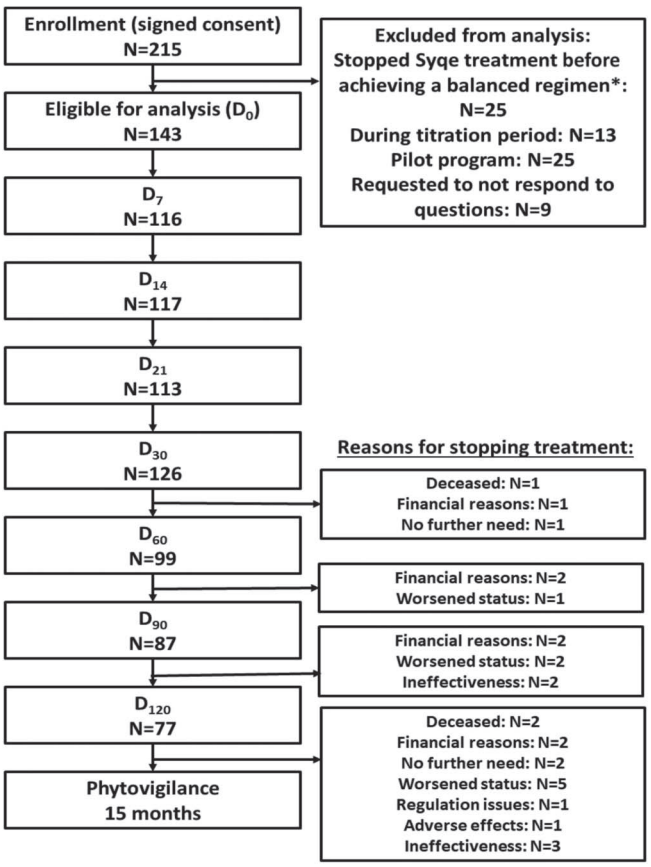

Figure 2. CONSORT 2010 flow diagram (numbers of patients). D, day from the first use of the inhaler; only patients who reported their NPS score were included in each visit's analysis; *, 25 patients (17%) did not achieve a balanced regimen because 1 patient (<1%) did not intend to use the inhaler a priori, 6 (4%) for financial reasons, 6 (4%) deceased (causes unrelated to treatment with the inhaler), 1 (<1%) improved health, 3 (2%) worsened health (causes unrelated to treatment with the inhaler), 3 (2%) stopped treatment because of adverse events, and 5 (3%) stopped treatment because of ineffectiveness.

3.2. Previous medical cannabis treatment

Seventy-five patients (52%) used MC before using the inhaler. The most common form of MC was oil extract (n = 38, 51%), followed by inhalation of inflorescence (n = 16, 21%). Twenty-one patients (28%) used both administration methods concomitantly. The most common cannabis dose was 20 grams per month (n = 54, 38%). A lower dose of 10 grams per month was used by 4 patients (3%), whereas higher doses of 30, 40, 50, and 60 grams per month were used by 8, 5, 3, and 1 patients, respectively.

Of the 75 patients who had previously used MC, 57 (76%) retrospectively reported experiencing MC-related AEs before using the inhaler, 34 of these 57 patients (60%) used oil extracts of MC, 7 (12%) smoked or vaporized and 16 (28%) used both concomitantly.

3.3. Inhaler treatment characteristics

At the time of data analysis, the patients were using the inhaler for an average of 9.0 ± 4.1 months. The duration of the titration phase was 26 ± 10 days. After titration, the average daily aerosolized Δ^9-THC dose was 1,500 ± 688 μg. After achievement of a stable dose, only a minority of the patients (n = 13, 9%) required rescue inhalations.

Of the 75 patients who had used MC before initiating treatment with the inhaler, 12 (16%) concomitantly continued their non-inhaler MC treatment. Nine of these 12 patients (75%) reduced their monthly MC doses.

3.4. Treatment effectiveness

The 5 patients who were not treated for chronic pain were not included in the effectiveness analysis.

At the end of the titration phase, 105 patients (76%) reported a reduction in pain intensity of at least 1 NPS point, 24 patients (17%) reported no change, and 9 patients (7%) reported an increase in pain intensity of at least 1 point. Comparison of the 33 patients who reported increased pain intensity or no change in pain intensity at the end of the titration period revealed no unique demographic or pain etiology characterization that could distinguish them from patients who responded to the treatment. Forty-eight patients (34%) and 16 patients (12%) reported a reduction of ≥30% and ≥50%, respectively, in pain intensity.

Among the 138 patients who had chronic pain, pain intensity measured by the NPS decreased by 1.62 points (95% CI, −1.99 to −1.24; $P < 0.001$)—from 7.3 ± 1.5 at baseline to 5.5 ± 1.6 at 120 days after treatment initiation (22.8% pain reduction). In the PP population, pain intensity significantly decreased by 2.6 points (95%CI, −2.9 to −2.2; $P < 0.001$)—from 7.3 ± 2.2 points at baseline to 5.1 ± 1.3 points at 120 days (25.4% pain reduction).

Among 67 patients with severe pain intensity at baseline (≥8 points), pain intensity significantly decreased by 2.1 points (95% CI, −2.6 to −1.7; $P < 0.001$)—from 8.4 ± 0.6 points at baseline to 6.0 ± 1.2 points at 120 days after treatment initiation (28.4% pain reduction **Fig. 3**). Of 43 patients who reported opioid use at baseline, 25 patients (58%) reported reduced opioid doses at 120 days after initiating treatment with the inhaler.

3.4.1. Evaluation of quality of life

Ninety-two of 143 patients (64.3%) rated the change in their QoL from initiation of treatment (**Fig. 4**). None of them reported "much worse" or "worse" QoL. Nine percent reported "no change," 59% reported "better," and 33% reported "much better" QoL.

3.5. Treatment safety

Among the 143 patients, 59 (41%) reported 102 treatment-related AEs (**Table 1**). The most common treatment-related AEs were dizziness (n = 26, 18.1% of patients), headache (n = 15, 10.7%), and sleepiness (n = 11, 7.7%). Most AEs (n = 66, 64%) were reported before the end of the titration phase, and less AEs were reported during the maintenance phase; the median day for AE reporting was −8 (ie, before the end of the titration period). Specifically, of the 143 patients in the ITT population, 48 (34%), 6 (4%), 6 (1%), and 2 (2%) reported at least 1 AE at 1, 3, 6, and 9 months, respectively, from treatment initiation. None of the patients reported AEs at 12 and 15 months. **Figure 5A** demonstrates the distribution of the 102 AEs across time, relative to the end of the titration period ("0" on the *x*-axis). Most AEs were short-termed, with a median of 15 minutes (IQR, 5–30 minutes) (**Fig. 5B**). All AEs resolved spontaneously without an intervention. None of the reported AEs were caused by malfunction of the inhaler.

Of the 59 patients who reported AEs during treatment with the inhaler, 30 (51%) had used MC before starting treatment with the inhaler and had retrospectively reported AEs from that previous MC use. The rate of AEs reported after MC administration with the

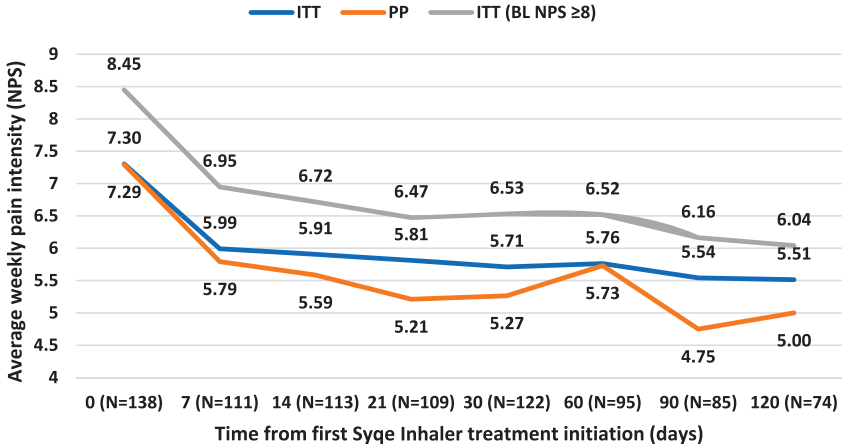

Figure 3. Changes in pain intensity during treatment. For the PP sample, N = 38 at all time points; for the ITT sample, baseline NPS ≥ 8, N = 67 at BL. BL, baseline; ITT, intention-to-treat population; N, number of patients on the *x*-axis represents the full ITT analysis sample; NPS, numeric pain scale; PP, per-protocol population.

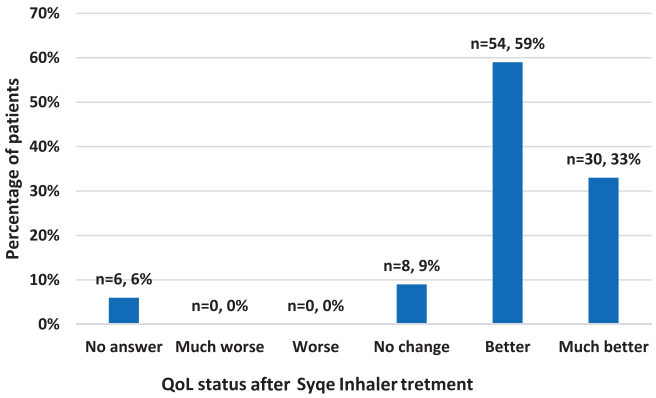

Figure 4. Changes in perceived quality of life after medical cannabis treatment using the Syqe Inhaler. The numbers on the bars indicate the number of patients who responded. N, number of patients; QoL, quality of life.

metered-dose inhaler rate was 35% lower than the rate of AEs reported when using MC by other administration routes (41% vs 76%).

4. Discussion

In the current study, we assessed the long-term effectiveness and safety of low and precise MC doses administered through the metered-dose Syqe Inhaler on patients with chronic pain due to various etiologies. Our analysis shows that after a structured titration phase, which was guided by professional nurses, patients reported significant pain reduction ranging from 22.8% in the ITT population to 28.4% in the population that reported high pain intensity at baseline of ≥8 points on the NPS. The percentage of reduction in pain intensity was comparable with the rate of 22.3% seen after 90 days of sublingual or smoked or vaped MC use.[4]

Table 1

Treatment-related adverse events.

Adverse events	Study population N = 143 n (%)
Total	59 (41)
Nervous system disorders	**33 (23)**
Dizziness	25 (17)
Confusion	3 (2)
Sleepiness	11 (8)
Concentration impairment	3 (1)
Memory impairment	2 (3)
Headache	15 (10)
Gastrointestinal disorders	**14 (10)**
Nausea	4 (3)
Heartburn	3 (2)
Dry mouth	6 (4)
Vomiting	1 (<1)
Psychiatric disorders	**15 (10)**
Anxiety	8 (6)
Restlessness	2 (1)
Cardiovascular disorders	**2 (1)**
Palpitations	2 (1)
Miscellaneous disorders	**18 (13)**
Cough	4 (3)
Tinnitus	1 (<1)
Muscle pain	1 (<1)

N do not add up to 100% because of concomitant adverse events. Bold values represent adverse effects rates by a systemic grouping.

Although 17% of the patients in the current study reported no decrease in pain intensity at the end of the titration phase and 7% reported worsening of pain, these patients elected to continue using the inhaler. It is possible that these patients have found other treatment benefits for using the inhaler, such as improved sleep or mood, which are often reported by patients treated with MC.[4]

The rate of AEs during the study was low, and most of them were reported during the titration phase, essentially disappearing after attainment of a stable treatment regimen. These results are in line with our previous clinical studies, in which significantly lower Δ^9-THC plasma levels were associated with similar analgesic effects and superior safety profiles compared with that observed with MC cigarette smoking.[4]

Although the AE rates of sublingual or smoked or vaped MC declined from 40% after a month of treatment to 30% a year later,[4] in the current study, AEs declined from 34% during the titration phase to almost none during the maintenance phase, which ranged from 3 to 15 months. In a study on patients with fibromyalgia treated by a single vaporizer session of MC from the Bedrocan cultivar, which is the cultivar used for the VCs of the inhaler, 80% of the treated patients reported psychoactive effects,[11] whereas in our study, only 10% reported psychoactive AEs, such as anxiety and restlessness. Hence, MC treatment using the inhaler seems similar to traditional MC for effectiveness, but superior regarding safety, while exposing the patients to very low doses of aerosolized Δ^9-THC.

The mean daily stable dose used by patients in the study was 1,500 μg (1.5 mg) of aerosolized Δ^9-THC, which is much lower compared with other administration routes of MC.[19] Two recent guideline articles recommended a maximal daily dose of 40 to 50 mg of Δ^9-THC through the oral or sublingual route.[8,18] Furthermore, the mean monthly amount of MC prescribed per patient in Israel (as of mid-2021) was about 30 gr, which equals about 1 gram per day. As the concentration of Δ^9-THC in MC supplied in Israel can vary between 1% and 20%, patients consume approximately 10 to 200 mg of Δ^9-THC daily.

The high Δ^9-THC doses of conventional MC use[19] produce high Δ^9-THC plasma levels (C_{max} range of ~50–250 ng/mL[17]), which are much higher than those required for achieving pain relief, and result in a higher rate of AEs.[1] Wallace et al.[22] showed that the therapeutic window for optimal pain reduction is 16 to 31 ng/mL of plasma Δ^9-THC.[22] Congruently, in a 3-arm randomized clinical trial using the Syqe Inhaler, administration of 500 μg and 1000 μg of aerosolized Δ^9-THC produced average C_{max} plasma Δ^9-THC levels of 14.3 ± 7.7 ng/mL and 33.8 ± 25.7 ng/mL, respectively. The range of this therapeutic window is further supported by the pharmacokinetics study on nabiximols oromucosal spray, where after the administration of 8 consecutive sprays, the maximal Δ^9-THC plasma concentration was 5.4 ± 2.41 ng/mL[21]—too low to show a clinically meaningful analgesic effect of this administration mode.[5] Furthermore, the inhaler enables repeatable dosing and structured titration of MC, which allow achieving a stable and consistent steady-state dosage compared with smoking or vaporizing. The latter shows considerable variability in plasma Δ^9-THC levels among patients and within each patient.[17] Hence, administration of MC with conventional administration routes may result in overdosing or AEs even after a prolonged duration of treatment.[3]

4.1. Limitations

This study has several limitations. First, the sample is relatively small. Second, because of the ongoing nature of the study design and the

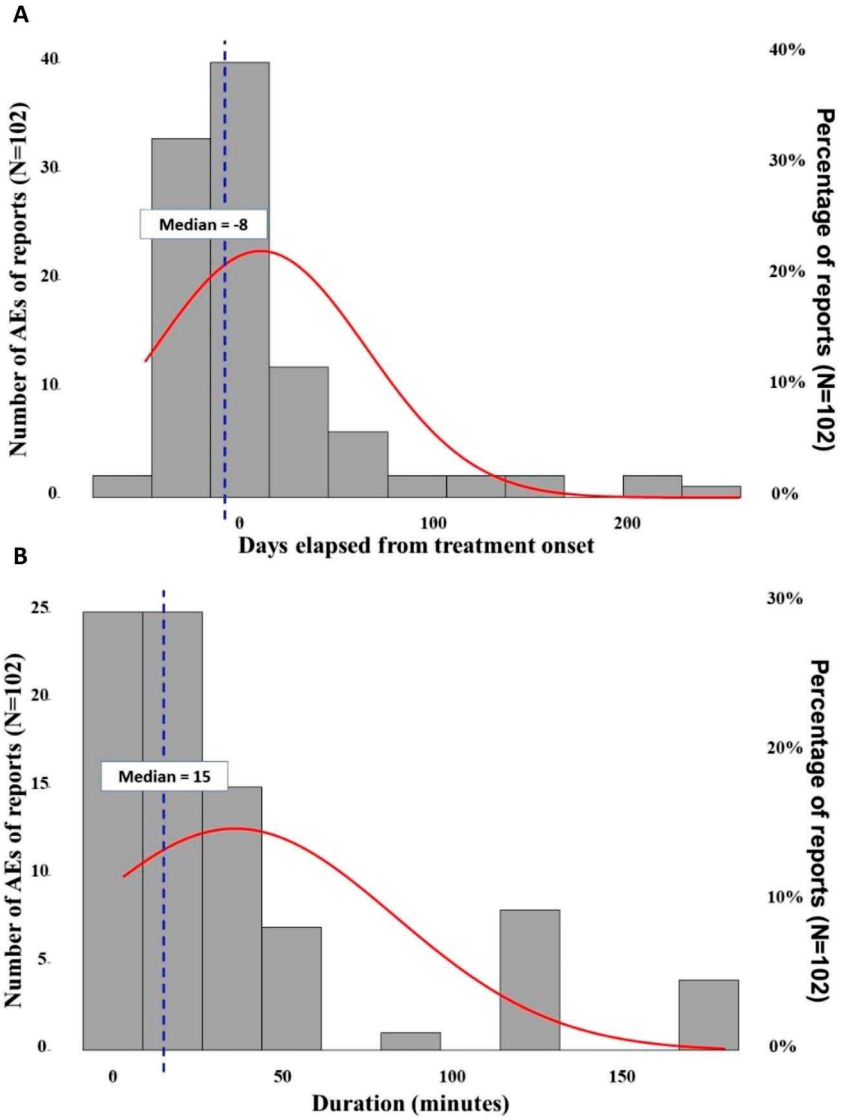

Figure 5. Characteristics of adverse events (AEs) reported after medical cannabis treatment using the Syqe Inhaler by time from treatment initiation and AE duration. (A) The time that elapsed from inhaler treatment onset to the report of AEs; "0" refers to the end of titration for each patient, relative to the time for which the AE was reported. (B) The duration (in minutes) of all reported AEs; red lines demonstrate the nonnormal (skewed) distribution of the measure, with most AEs reported during the titration period and not after stabilization (5A). Most AEs were short-termed (5B).

19.5% attrition rate, the sample size became smaller at each time point; however, the PP analyses controlled for this limitation.

5. Conclusions

Medical cannabis treatment with the Syqe Inhaler demonstrated overall long-term pain reduction, quality of life improvement, and opioid-sparing effect in a cohort of patients with chronic pain, using just a fraction of the amount of MC compared with other modes of delivery by inhalation. These outcomes were accompanied by a lower rate of AEs and almost no AE reports during a long-term steady-state follow-up. Additional follow-up in a larger population is warranted to corroborate our findings.

Disclosures

J. Aviram and D. Atzmony are employed by Syqe Medical LTD. E. Eisenberg is employed by Syqe Medical Ltd as an external consultant.

Syqe Medical Ltd funded the study fully.

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PR9/A162.

Article history:
Received 10 January 2022
Received in revised form 7 April 2022
Accepted 11 April 2022

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Journal of Cannabis Research

ORIGINAL RESEARCH

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THC degradation does not impair the accuracy of THC doses aerosolized by the metered-dose SyqeAir inhaler: a 24-month stability trial

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Abstract

Background: Although the worldwide use of medical cannabis (MC) is on the rise, there is insufficient data regarding the long-term stability of phytocannabinoids in the plant material under different storage conditions. Specifically, there is insufficient data on the effect of storage conditions on the availability of (-)-Δ^9-trans-tetrahydrocannabinol (THC) in vaporized cannabis. The Syqe inhaler delivers metered doses of phytocannabinoids by inhalation and utilizes accurate quantities of ground cannabis inflorescence packaged in tamper-proof cartridges. We aimed to assess the stability of phytocannabinoids in ground cannabis before and after packaging in Syqe cartridges as well as the reproducibility of THC delivery in the aerosolized dose.

Methods: Ground MC inflorescence was stored under different temperature and humidity conditions, before or after being packaged in Syqe cartridges. Concentrations of the major phytocannabinoids therein were analyzed at different time points using ultra-high performance liquid chromatography (U-HPLC). THC doses aerosolized via the Syqe inhaler were evaluated using cartridges stored for up to 2 years at 25°C. Every vapor chip contains 13.5±0.9 mg of ground MC powder.

Results: No significant changes were observed in phytocannabinoid concentrations in ground cannabis inflorescence after 3 months of bulk storage in a polypropylene container and sealed in an aluminum foil pouch at 5°C. In contrast, significant changes in phytocannabinoid concentrations were found when ground inflorescence was stored in the cartridges at 25°C for 2 years. Specifically, CBGA, THCA, and total THC concentrations decreased from 0.097±0.023, 2.7±0.3, and 2.80±0.16 mg/chip at baseline to 0.044±0.007 (55% decrease), 1.50±0.27 (44% decrease), and 2.20±0.083 (21% decrease) mg/chip following 2 years, respectively, while CBN and THC concentrations increased from 0.005±0.005 and 0.44±0.11 mg/chip at baseline to 0.14±0.006 (2700% increase) and 0.88±0.22 (100% increase) mg/chip following 2 years, respectively. Storage at 30°C revealed a steeper change in phytocannabinoid concentrations within an even shorter period. Despite the significant change of relative cannabinoid composition within the cartridge, the actual THC dose present in the aerosol remained relatively stable throughout this period and within the dosage range of 500mcg±25% required for pharmaceutical-grade inhalers.

Conclusions: MC powder in Syqe cartridges may be stored at room temperature for at least 2 years after production without affecting the aerosolized THC dose delivered to patients by more than ±25%. Future studies should analyze

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additional phytocannabinoids and terpenes in the cannabis inflorescence and assess the stability of different cannabis cultivars following storage in Syqe cartridges.

Keywords: Phytocannabinoids, Medical cannabis, Stability, Inhalation

Introduction

The interest of Western modern medicine in the therapeutic potential of *Cannabis sativa* L. (*Cannabis*) started following the report of O'Shaughnessy in his 1839 book on the therapeutic effects of Indian hemp (O'Shaughnessy, 1839). Since then, despite a long period of *Cannabis* prohibition (David et al., 2014), treatment of many clinical indications using medical cannabis (MC) has been on the rise (Boehnke et al, 2016). In recent decades, research on *Cannabis* has significantly advanced and, thus far, 144 phytocannabinoids were identified (Berman et al, 2018). The main natural phytocannabinoids are (-)-Δ^9-trans-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabigerolic acid (CBGA). As these compounds contain a chemically unstable carboxyl group (COOH), when heat is applied they are converted by a process known as decarboxylation into (-)-Δ^9-trans-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG), respectively (Peschel, 2016).

Another compound, cannabinol (CBN), which is formed by THC oxidation and by cannabinolic acid (CBNA) decarboxylation, has been established as a marker for *Cannabis* aging.

Cannabis cultivars are typically classified by their THC and CBD concentrations (Peschel, 2016). THC-rich cultivars are designated as type I, CBD-rich cultivars as type III, and cultivars with comparable concentrations of THC and CBD as type II (Russo, 2011). This categorization system is currently the basis of most clinical treatments using the *Cannabis* plant (Aviram et al, 2020a) in countries where MC use has been legalized.

MC is mostly administered via smoking or vaporization (Hazekamp et al, 2013; Aviram et al, 2020b) but these administration routes are not metered or accurate (Huestis, 2009). To overcome the problem of inaccurate dosing, the Syqe metered dose inhaler (Trade name: SyqeAir inhaler, Syqe Medical Ltd., Tel Aviv, Israel) was developed. The inhaler is a battery-operated, handheld, thermal-selective metered-dose inhaler that utilizes a tamper-proof cartridge containing vapor chips (VCs). Each VC holds a precise quantity of processed medical-grade *Cannabis* powder from dried natural *Cannabis* inflorescences manufactured under clean-room conditions (Lai et al, 2005). The inhaler is preprogrammed with several distinct heating protocols, each tailored to heat a VC and deliver an aerosol containing 250, 500, 750, or 1000 mcg of THC to the patient. The inhaler's settings can be selected at the press of a button. In operation, the inhaler heats the MC to a temperature below combustion and engages automatic thermal and airflow controls that ensure precise, accurate, and high-efficiency delivery of the selected dose of MC aerosol to the patient's lungs, independent of the patients' inhalation pattern. The patient has no direct contact with the MC, and only inhales phytocannabinoids vaporized there from. The device requires minimal training prior to use and automatically generates logs of the patient treatment regimen (dose × time of day) that connects to an app on the patient smartphone, so that the patient as well as the treating physician can have information regarding the patient's actual adherence to a prescribed regimen and thereby learn to optimize the treatment over time.

Currently, THC is used for dose selection (e.g., 500 mcg THC) and it serves as an indicator for the amounts of other molecules, such as minor phytocannabinoids, terpenes, and more that vaporize into the aerosol during the vaporization of a selected THC dose. In recent years, Syqe inhaler technology was evaluated in several clinical trials (Eisenberg et al, 2014; Vulfsons et al, 2020; Almog et al, 2020) and in a long-term observational study (Aviram et al, 2022), where it demonstrated effectiveness, safety, and usability as well as narrow pharmacokinetic variability.

Milay et al. (2020) investigated the optimal storage conditions for preserving the composition of naturally occurring secondary metabolites (i.e., phytocannabinoids and terpenoids) in *Cannabis* inflorescences and extracts post-harvest over a 1-year period. They reported significant variability in the stability of the main phytocannabinoids at room temperature (25°C) and concluded that the best conditions for preserving the original phytocannabinoid and terpene contents of inflorescences throughout long storage periods are in the form of whole (non-ground) inflorescences at 4°C (Milay et al, 2020). In the current study we aimed to assess the stability of these phytocannabinoids during the manufacturing process (as will be described in the Methods section) at the Syqe Medical facility and within the vacuum-sealed cartridges for a period of 2 years. In addition, we assessed whether storage time has an effect on the dose of THC aerosolized via the Syqe inhaler from such cartridges. In this stability study, only the 500 mcg THC dose was assessed as it was found to be the optimal dose for chronic pain treatment

in a previous study, with the best balance between pain intensity reduction report and intoxication levels (Almog et al, 2020).

Methods

Chemicals and reagents

Liquid chromatography LiChrosolv® gradient grade acetonitrile, methanol, and water for the mobile phase were purchased from Mercury Scientific and Industrial Products Ltd. (Rosh Haayin, Israel). Liquid chromatography-mass spectrometry (LC-MS)-grade formic acid was purchased from BioLab Ltd. (Jerusalem, Israel). Phytocannabinoids analytical standards (>98% purity) of THC, THCA, CBD, CBDA, CBN, and CBGA were manufactured by Restek (Bellefonte, PA, USA). Data were restricted to the requirements of the Israeli Medical Cannabis Authority regulations.

Medical cannabis

The MC inflorescence product "Bedrocan" (Bedrocan International, Veendam, Netherlands), which contains about 22% THC and <1.0 CBD, was used in all experiments. It was free of pesticides, heavy metals (<0.2 ppm lead, <0.02 ppm mercury, and <0.02 ppm cadmium), stalks, and foreign materials. Microbiological purity was also confirmed by the manufacturer (total aerobic microbial count of <10 colony forming units [CFU]/g, total yeast and mold count of <10 CFU/g, and absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and bile-tolerant gram-negative bacteria).

MC processing, cartridge production, handling, and storage

Before processing, the MC powder is stored at 25°C (± 2°C) and relative humidity (RH) of 60% (± 5%) in a polypropylene container, sealed in an aluminum foil pouch (Oliver-Tolas Healthcare Packaging B.V., Venray, the Netherlands). All MC handling — starting from processing until a cartridge is vacuum sealed — are performed in a clean-room environment. Manufacture begins with the grinding of whole *Cannabis* inflorescences (Bedrocan MC inflorescence product) to form a fine and homogenized powder (particle diameter <1000 μm).

VCs are manufactured by a dedicated machine, which allots 13.5±0.9 mg of ground MC powder into each VC. Sixty VCs are subsequently assembled into each tamper-proof cartridge which is then packaged in a vacuumed aluminum foil that protects it from exposure to light and oxygen until use. The Syqe inhaler releases a single VC for each inhalation and returns it to the cartridge after use. To ensure dose accuracy, each VC is used by the inhaler only once. In this stability study, only the 500 mcg THC dose was assessed.

Assessment of pre-packaging MC stability

To assess the effect of refrigeration (5°C±2°C with up to 12% RH) on its stability during the handling and hold time, samples were taken from three separate MC powder batches and assayed at baseline (BL) and at 1, 2, and 3 months after grinding. Each sample was assayed for loss on drying and for the concentration of the major phytocannabinoids (CBDA, CBD, CBGA, CBN, THCA, and THC; ordered according to the ultra-high-performance liquid chromatography (U-HPLC) test).

Long-term stability of MC stored in cartridges

Once cartridges are produced, they are stored, distributed, and used at room temperature. To assess the shelf life of such cartridges under those conditions, a long-term stability study was performed. To that end, cartridges were stored at 25°C (± 2°C) and relative humidity of 60% (± 5%) in a calibrated and certified stability chamber (Memmert Constant climate chamber HPP260). Batches were tested at BL (n=9) and after 3 (n=6), 6 (n=9), 9 (n=8), 12 (n=7), 18 (n=4), and 24 (n=4) months. At each time point, samples of the ground inflorescence within the cartridges were analyzed for loss on drying and for the relative amount (mg) of each of the major phytocannabinoids (CBDA, CBD, CBGA, CBN, THCA, and THC). Cartridges from the same batches were also used for aerosolization by the Syqe inhaler at BL and at 6, 9, 18, and 24 months, and the THC content of the aerosolized dose was analyzed.

To assess the effects of a warmer temperature on MC, cartridges were stored post-production at an elevated temperature of 30°C (±2°C) and relative humidity of 65% (± 5%). Three different batches were analyzed at BL and at 1, 2, 4, and 6 months of storage.

Chemical analysis of phytocannabinoids

To analyze the six main phytocannabinoids (CBDA, CBD, CBGA, CBN, THCA, and THC) of *Cannabis* inflorescences, 100 mg of ground inflorescences from the same container bulk were weighed in duplicates and combined with 25 mL methanol. The samples were extracted for 40 min in an ultrasonic bath and centrifuged at 3000 rpm for 5 min. After dilution in 70/30 ACN/water + 0.1% formic acid solution, the samples were filtered through a 0.22 μm PP filter vial. The phytocannabinoids were analyzed by an U-HPLC system with a refrigerated auto-sampler, thermostatic column oven, and ultraviolet detector (Waters ACQUITY UPLC H-Class, Waters Corporation, Milford, MA, USA). Chromatographic separation was performed using a Waters ACQUITY UPLC 1.7-μm C18 column 2.1 × 150 mm, maintained at 30°C. The phytocannabinoids were separated using gradient elution with

0.1% (v/v) formic acid in double-distilled water (phase A) and 0.1% (v/v) formic acid in acetonitrile (phase B). A constant flow rate of 0.4 mL/min was employed throughout the analysis. The gradient profile varied from 70% to 100% B in 10.5 min, held for 0.5 min in these conditions, and returned to the initial conditions. The runtime was 14 min. Quantitation of the samples was performed according to calibration curves.

For THC and CBD, the concentrations of the acid and its neutral counterpart were summed and reported as the total content. For example, the concentration of total THC was calculated as total THC $=(\text{THCA} * 0.877) + \text{THC}$, with 0.877 being the molar ratio between the two compounds that correct for a change in the mass of THCA due to the loss of CO_2 (decarboxylation). Figure 1 is an example of a baseline U-HPLC analysis of the Bedrocan MC inflorescence product.

Loss on drying

To determine the amount of volatile matter (reported as loss on drying content) in the sample 1.1g ± 0.1g of mixed MC flowers sample or ground powder was weighted and spread in a thin layer over the moisture analyzer (MA100, Sartorius AG, Göttingen, Germany) sample pan. The test was performed in duplicates at 90°C for 10/20 min for ground MC powder or MC flowers raw material, respectively.

Statistical analysis

The R software package (V.1.1.463) with tidyverse (Tidyverse, 2019) was used to analyze changes in outcome measures by one-way analysis of variance (ANOVA), using Fisher's exact test. All analyses were followed by a Tukey post hoc test for multiple comparisons. Data are presented as mean and 95% confidence interval (CI). Differences were considered significant at the $p<0.05$ level.

Results

The effect of refrigeration on the phytocannabinoid contents of ground *Cannabis* inflorescence prior to packaging

A significant increase in loss on drying from 5.6±0.06% at BL to 6.6±0.29% at 3 months ($F_{(3,8)}=21.56$; $p<0.001$) was observed (Fig. 2A). Tukey post hoc test showed a statistically significant difference between loss on drying levels at BL and at 3 months ($t_{(8)} = -7.53$; $p<0.001$), at 1 month compared to 3 months ($t_{(8)} = -5.93$; $p<0.01$) and at 2 months compared to 3 months ($t_{(8)} = -3.43$; $p<0.05$).

As shown in Fig. 2B–H, no significant change was detected during the study in any of the phytocannabinoids: CBDA ($F_{(3,8)}=2.58$, $p=0.13$), CBGA ($F_{(3,8)}=0.06$, $p=0.97$), CBN ($F_{(3,8)}=0.31$, $p=0.81$), THC ($F_{(3,8)}=0.006$, $p=0.99$), THCA ($F_{(3,8)}=0.03$, $p=0.99$), Total THC ($F_{(3,8)}=0.29$, $p=0.82$), and total CBD ($F_{(3,8)}=2.59$, $p=0.12$). As expected, due to the baseline concentrations

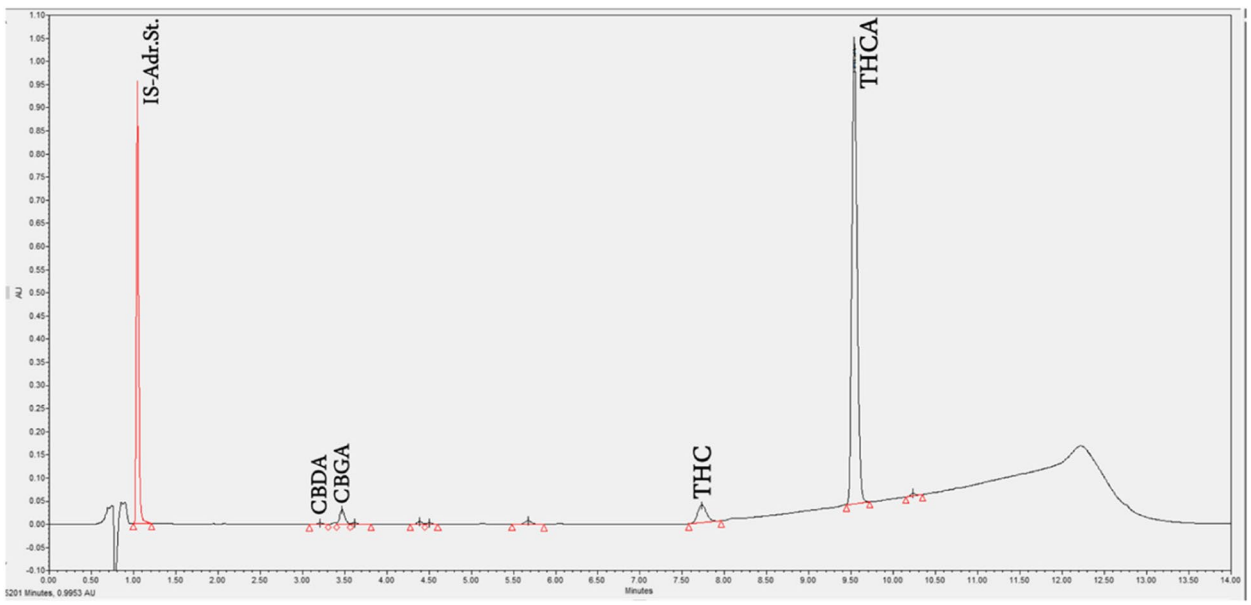

Fig. 1 U-HPLC analysis of the Bedrocan MC inflorescence product. From left to right, the analyzed phytocannabinoids were CBDA, CBGA, THC, and THCA. CBN and CBG are undetectable in a fresh specimen. CBDA, cannabidiol acid; CBGA, cannabigerolic acid; THC, (-)-Δ^9-trans- tetrahydrocannabinol; THCA, (-)-Δ^9-trans- tetrahydrocannabinol acid; CBN, cannabinol; CBG, cannabigerol

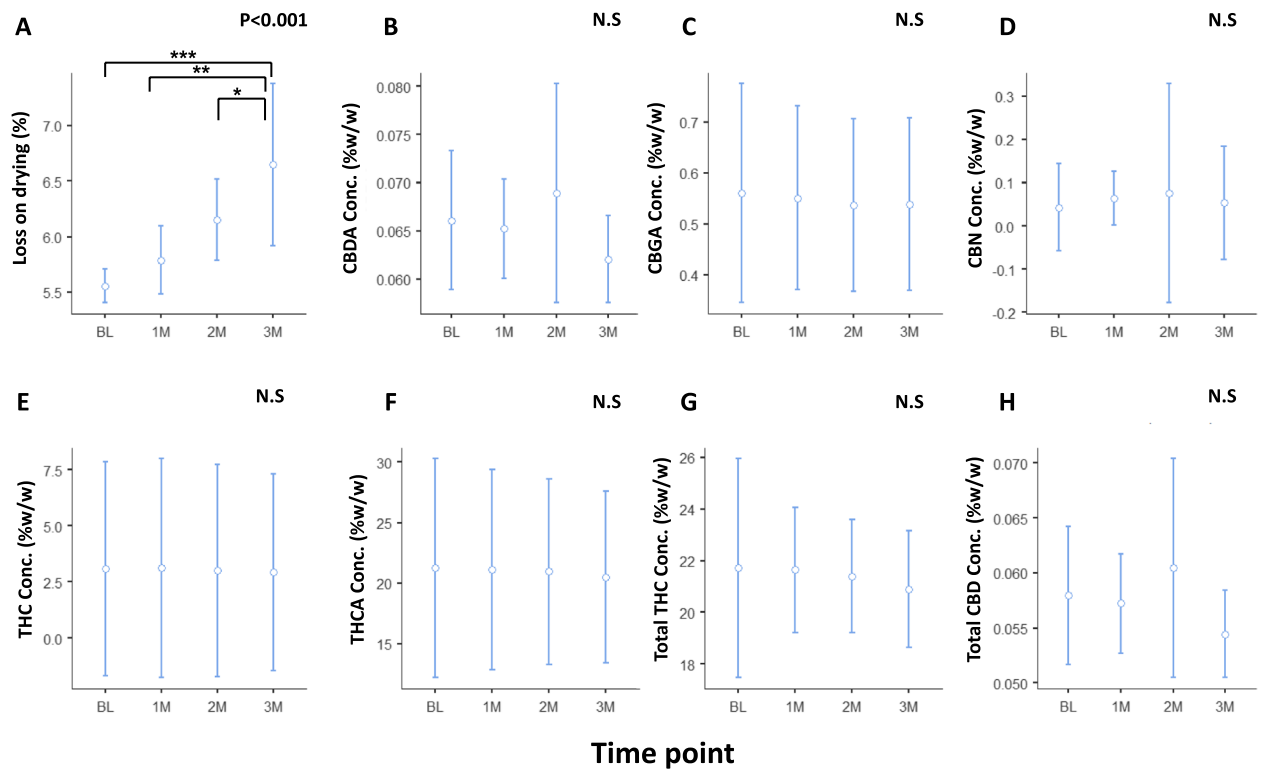

Fig. 2 Concentration of major phytocannabinoids in pre-packaged ground *Cannabis* inflorescence stored at 5°C. The middle circle represents the mean value of several duplicates and the upper and lower lines represent the upper and lower 95% confidence interval values. BL, baseline; M, month; CBDA, cannabidiol acid; THC, (-)-Δ^9-trans- tetrahydrocannabinol; THCA, (-)-Δ^9-trans- tetrahydrocannabinol acid; CBGA, cannabigerolic acid; CBN, cannabinol; "Total" represents the sum of the acid and neutral components. Statistically significant differences between times of storage were calculated by one-way ANOVA, utilizing Fisher's exact test analyses (whenever significant, stated as the *p* value and when not significant stated as N.S.), followed by a Tukey post hoc multiple comparison test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)

of CBD in this cultivar (<1%), CBD concentrations were mostly undetected at all time points. These results confirm that the conditions in which the ground inflorescences are preserved maintain stable phytocannabinoid concentrations.

The effect of storage parameters on phytocannabinoid concentrations in ground *Cannabis* inflorescence packaged in Syqe cartridges

To assess the effect of storage at room temperature on phytocannabinoids content in ground *Cannabis* in Syqe cartridges, a 2-year stability study was performed.

As shown in Fig. 3, no significant changes were observed in loss on drying ($F_{(6,31)}=0.68$, $p=0.66$), in CBDA content ($F_{(6,40)}=1.90$, $p=0.10$) and total CBD content $F_{(6,37)}=0.67$, $p=0.67$). In contrast, significant decreases were observed in CBGA ($F_{(6,40)}=8.96$, $p<0.001$), THCA ($F_{(6,40)}=11.26$, $p<0.001$), and total THC contents ($F_{(6,40)}=9.94$, $p<0.001$). Tukey post hoc test showed a significant decrease in CBGA content between BL and 24 months ($t_{(40)}=5.33$, $p<0.001$) and between 3 months and 24 months ($t_{(40)} = -4.07$, $p<0.01$). THCA content decreased significantly between BL and 24 months ($t_{(40)}=6.72$, $p<0.001$), 3 months and 24 months ($t_{(40)}=4.79$, $p<0.001$), 6 months and 24 months ($t_{(40)}=3.96$, $p<0.01$), and between 9 months and 24 months ($t_{(40)}=3.54$, $p<0.05$). Total THC content decreased significantly between BL and 24 months ($t_{(40)}=5.83$, $p<0.001$), 3 months and 24 months ($t_{(40)}=4.55$, $p<0.001$), 6 months and 24 months ($t_{(40)}=3.56$, $p<0.05$), and between 9 months and 24 months ($t_{(40)}=3.17$, $p<0.05$).

CBN and THC content increased significantly ($F_{(6,40)}=122.18$, $p<0.001$ and $F_{(6,40)}=6.04$, $p<0.001$, respectively). Notably, CBN amounts increased between BL and 24 months ($t_{(40)} = -22.18$; $p<0.001$), 3 months and 24 months ($t_{(40)} = -18.17$, $p<0.001$), 6 months and 24 months ($t_{(40)} = -17.08$, $p<0.001$), 9 months and 24 months ($t_{(40)} = -14.82$, $p<0.001$), 12 and 24 months ($t_{(40)} = -11.40$, $p<0.001$), and between 18 and 24 months ($t_{(40)} = -3.84$, $p<0.01$). THC content increased between BL and 24 months ($t_{(40)} = -5.40$, $p<0.001$) and between

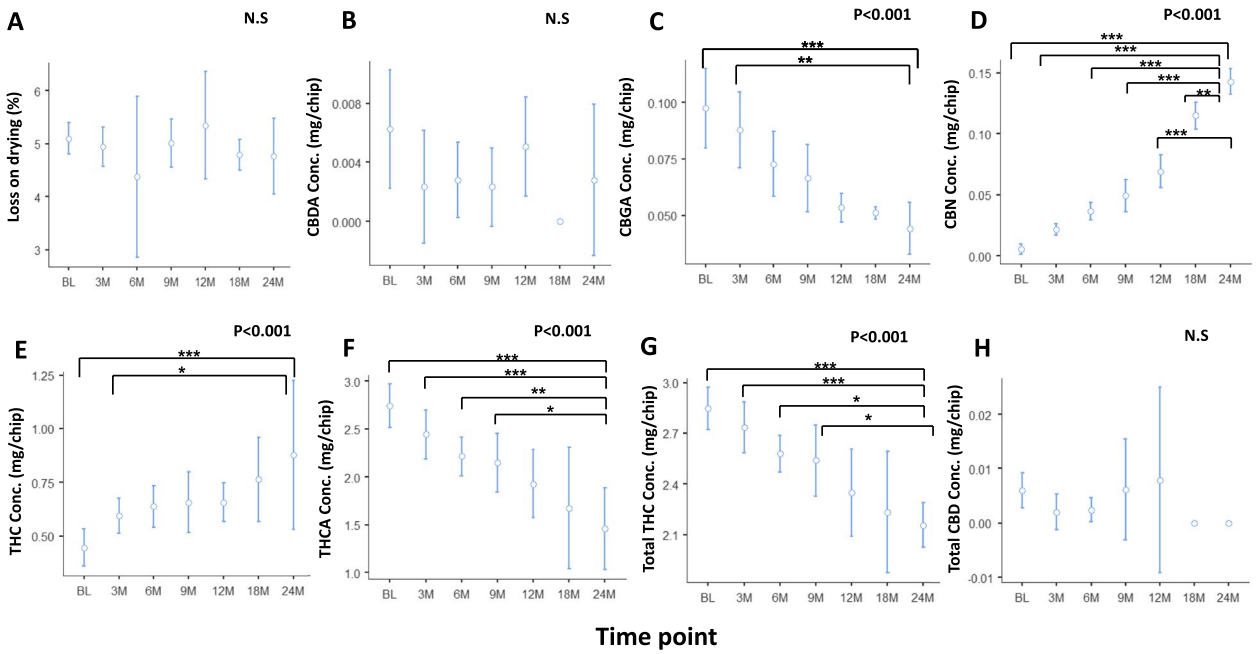

Fig. 3 Amount of major phytocannabinoids in MC stored in Syqe cartridges at room temperature over 2 years. The middle circle represents mean value and the upper and lower lines represent the upper and lower 95% confidence interval values. BL, baseline; M, month; CBDA, cannabidiol acid; THC, (-)-Δ^9-trans- tetrahydrocannabinol; THCA, (-)-Δ^9-trans- tetrahydrocannabinol acid; CBGA, cannabigerolic acid; CBN, cannabinol; "Total" represents the sum of the acid and the neutral components. Statistically significant differences between times of storage were calculated by one-way ANOVA, utilizing Fisher's exact test analyses (whenever significant, stated as the *p* value and when not significant stated as N.S.), followed by a Tukey post hoc multiple comparison test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)

3 months and 24 months ($t_{(40)} = -3.29$. $p<0.05$). As expected, CBD was mostly undetected at all time points, as the pre-storage amount of its precursor, CBDA, was 0.006 mg/sample or less.

Analysis of phytocannabinoid content following storage of the Syqe cartridges at an elevated temperature of 30°C, showed that loss on drying values did not vary significantly over time ($F_{(4,12)}=1.12$, $p=0.19$), but CBGA, THCA, and total THC content decreased significantly ($F_{(4,12)}=33.57$, $p<0.001$; $F_{(4,12)}=22.52$, $p<0.001$; and $F_{(4,12)}=6.46$, $p<0.01$, respectively). Tukey post hoc test showed a statistically significant decrease in CBGA content between BL and 6 months ($t_{(12)}=11.17$, $p<0.001$), 1 and 6 months ($t_{(12)}=5.68$, $p<0.001$), 2 and 6 months ($t_{(12)}=6.76$, $p<0.001$), and between 4 and 6 months ($t_{(12)}=3.55$, $p<0.05$). A statistically significant decrease in THCA content was observed between BL and 6 months ($t_{(12)}=8.39$, $p<0.001$), 1 and 6 months ($t_{(12)}=5.00$, $p<0.01$), and between 2 and 6 months ($t_{(12)}=3.21$, $p<0.05$). Total THC content decreased significantly between BL and 6 months ($t_{(12)}=4.36$, $p<0.01$). Additionally, CBN and THC content increased significantly ($F_{(4,12)}=106.72$, $p<0.001$ and $F_{(4,12)}=44.44$, $p<0.001$, respectively). Tukey post hoc test showed a significant increase in CBN amount between BL and 6 months ($t_{(12)} = -19.26$, $p<0.001$), 1 and 6 months ($t_{(12)} = -13.30$, $p<0.001$), 2 and 6 months ($t_{(12)} = -10.79$, $p<0.001$), and between 4 and 6 months ($t_{(12)} = -5.66$, $p<0.001$). THC content increased between BL and 6 months ($t_{(12)} = -11.81$, $p<0.001$), 1 and 6 months ($t_{(12)} = -7.52$, $p<0.001$), and between 2 and 6 months ($t_{(12)} = -3.51$, $p<0.05$) (Fig. 4). As expected, CBD, CBDA, and total CBD content were mostly undetected at all time points and could not be analyzed.

Stability of aerosolized THC dose via the Syqe inhaler over 2 years of cartridges shelf life

In the framework of the 2-year stability study, THC dose was analyzed following aerosolization via the Syqe inhaler. The Syqe inhaler was set to aerosolize 500 mcg THC doses from the standard amount of MC contained within the VCs. Data are shown only for time points in which three or more batches were tested (namely, BL and 6, 9, 18, and 24 months). As shown in Fig. 5, contrary to the changes in the phytocannabinoid amounts in the ground inflorescences inside the cartridges, aerosolized THC doses remained relatively stable, but with some statistically significant changes over time ($F_{(4,15)}=9.09$, $p<0.001$). BL, 6-, 9-, 18-, and 24-months' values were 484.76±31.28 mcg, 486.30±50.56 mcg, 563±22.06 mcg, 597.94±16.75 mcg, and 554.67±36.00 mcg, respectively.

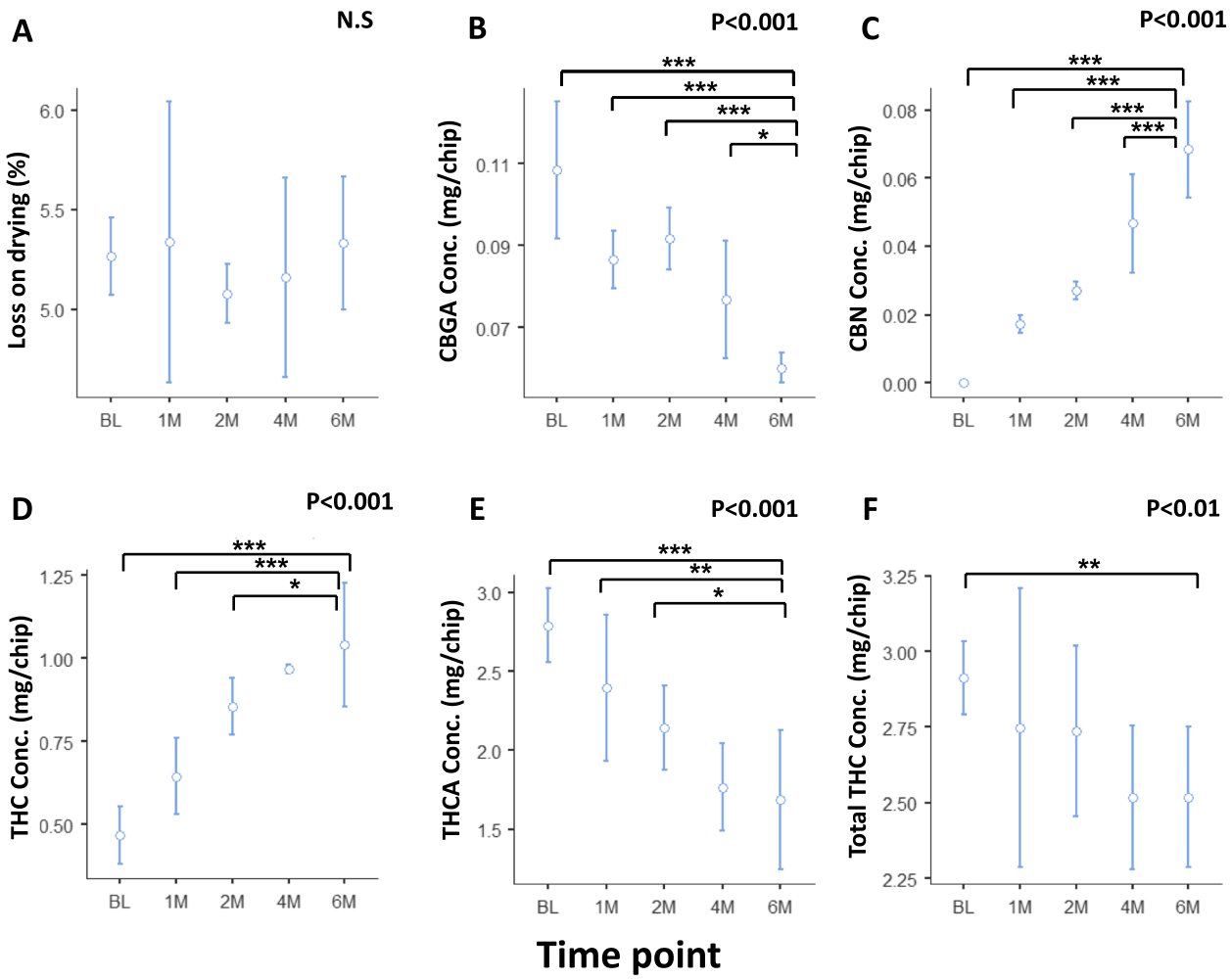

Fig. 4 Amount of major phytocannabinoids in MC stored in Syqe cartridges at 30°C over 6 months. The middle circle represents the mean value and the upper and lower lines represent the upper and lower 95% confidence interval values. BL, baseline; M, month; THC, (-)-Δ^9-trans- tetrahydrocannabinol; THCA, (-)-Δ^9-trans- tetrahydrocannabinol acid; CBGA, cannabigerolic acid; CBN, cannabinol; "Total" represents the sum of the acid and the neutral components. Statistically significant differences between times of storage were calculated by one-way ANOVA, utilizing Fisher's exact test analyses (whenever significant, stated as the *p* value and when not significant stated as N.S), followed by a Tukey post hoc multiple comparison test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)

All aerosolized THC doses were well within the 500 mcg ±25% range (i.e., 375–625 mcg) that apply to pharmaceutical-grade inhalers worldwide (18). Tukey's post hoc test did not show any significant changes between the time points analyzed.

Discussion

Our analysis did not find significant changes in the main phytocannabinoid concentrations in ground *Cannabis* after 3 months of bulk storage in a polypropylene container and sealed in an aluminum foil pouch at 5°C. This suggests that grinding of the bulk product may be performed at least up to 3 months prior to cartridge manufacture, without a detectable effect on phytocannabinoid composition in ground *Cannabis* inflorescence.

In contrast, significant changes in phytocannabinoid contents were found when the ground inflorescence stored in the cartridges at 25°C over a 2-year period. Specifically, CBGA, THCA and total THC concentrations decreased, while CBN and THC concentrations increased. These findings are in line with those reported by Milay et al. (2020), who reported that a THC-rich cultivar (a cannabis cultivar different than the cultivar of the current study) stored at room temperature for a period of 1 year demonstrated an increase in THC and CBN concentrations, reflecting THC generation due to decarboxylation of THCA by heat and light and simultaneous THC

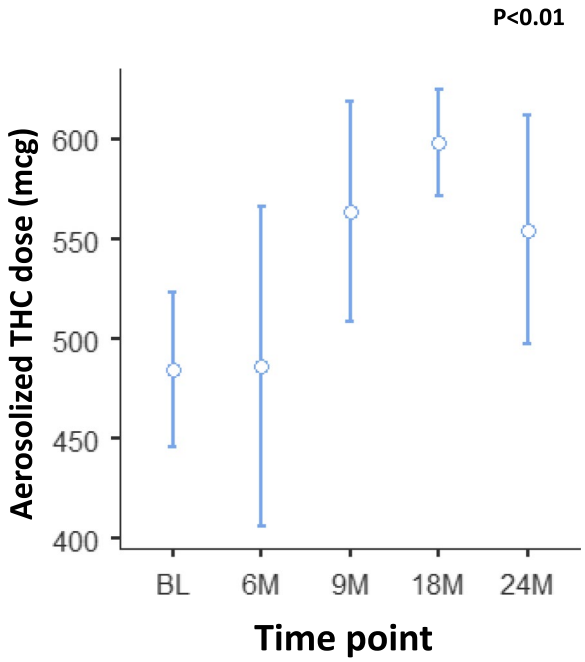

Fig. 5 Aerosolized THC doses after storage of MC in Syqe cartridges at room temperature over 2 years. The middle circle represents the mean value and the upper and lower lines represent the upper and lower 95% confidence interval values. BL, baseline; M, month; THC, (-)-Δ^9-trans- tetrahydrocannabinol. Statistically significant differences between times of storage were calculated by one-way ANOVA, utilizing Fisher's exact test analyses (whenever significant, stated as the *p* value and when not significant stated as N.S.), followed by a Tukey post hoc multiple comparison test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)

loss by degradation to CBN (Milay et al., 2020). Mentionable, in this comprehensive study, the authors did not perform analyses for THC doses that could be aerosolized at different time points of the analysis. In other words, even though the relative amounts of THCA, THC, and CBN could change during the storage of cannabis, it was not known if this may impact the actual amount of THC that would be released upon aerosolization of these inflorescences.

Although the decrease in THCA and total THC amounts in ground inflorescence stored in the vacuum-sealed cartridges were expected to be correlated with a respective decrease in aerosolized THC doses, these remained within the 500 mcg ±25% range (i.e., 375–625 mcg) as required for pharmaceutical grade inhalers (The United States Pharmacopeial Convention, 2012). This 25% variability range was recommended by the US Pharmacopeial Convention in 2012 for metered-dose inhalers and dry powder inhalers. It should be mentioned that the Syqe inhaler is designed to adhere to pharmaceutical requirement as much as possible in order to provide patients with a consistent aerosolized THC doses that provide consistent THC plasma levels (Eisenberg et al., 2014), and consistent short-term (Almog et al., 2020) and long-term clinical outcomes (Aviram et al., 2022). Syqe has the ambition to become a fully reliable, data-supported, and standardized administration form for inhaled cannabinoids, despite the fact that according to the currently available literature no other MC product currently available in the market follows any clear constraints or requirements on aerosolized THC doses and/or their variability range.

The apparent discrepancy between the decrease in THCA and total THC amounts in ground inflorescence and the relative stability amounts of aerosolized THC doses may be attributed to the increased concentration of THC as well as to the increased proportion of THC/THCA. We hypothesize as follows: The heating process of the Syqe inhaler generates THC-containing aerosol by two mechanisms: decarboxylation (of THCA to generate THC) and vaporization (of THC and THCA). The amount of THC in the aerosol is the sum of vaporized THC and vaporized and decarboxylated THCA. If the efficiency of THC vaporization is greater than that of the vaporization and decarboxylation of THCA then an increase in THC concentration in the plant would more than compensate for an equal loss in THCA concentration. Such overcompensation may explain the observed relative stability of aerosol THC despite a loss of total THC, as observed during storage at 25°C.

Storage of MC in the cartridges at an elevated temperature of 30°C demonstrated a more rapid degradation and decarboxylation process to an extent that would shorten the shelf life of the product. These results show that such temperatures should be avoided during pharmaceutical grade *Cannabis* production, storage, and transport. Furthermore, patients should be encouraged to keep the cartridges at lower temperatures whenever possible.

Our findings suggest that storage at 5°C suffices to maintain phytocannabinoid concentration in MC powder packaged as detailed in this study for up to 3 months. Accordingly, storage by freezing may be unnecessary for this phase of cartridge manufacture, thereby potentially simplifying the logistics of the production process, and reducing its costs. Once a cartridge is produced, it may be kept at room temperature or at a lower temperature to maintain a shelf life of at least 2 years without a significant effect on the dose delivered by aerosolization. Refrigeration may be unnecessary at this stage, potentially simplifying the distribution and storage requirements for the entire supply chain, as well as being more convenient to patients. It is nonetheless important to avoid significant exposure of cartridges to a temperature significantly higher than 25°C, in order to prevent the reduction in phytocannabinoids content.

Conclusions

Although significant changes in phytocannabinoid amounts occurred during extended storage (up to 2 years) of cartridges containing ground Bedrocan MC inflorescence, the therapeutic aerosolized THC dose remained relatively stable, within the dosage range of 500 mcg ±25% required for pharmaceutical-grade inhalers. This precise aerosolized THC dose, which have yet to be demonstrated in scientific studies by other devices or when *Cannabis* inflorescence is smoked in a cigarette — with or without a tobacco additive — provides an important improvement to the MC field. Future studies should analyze a much wider range of known phytocannabinoids and terpenes present in the *Cannabis* inflorescence and assess the stability of different *Cannabis* cultivars following storage in Syqe cartridges.

Abbreviations

THC: (-)-Δ^9-Trans-tetrahydrocannabinol; THCA: (-)-Δ^9-Trans-tetrahydrocannabinolic acid; CBD: Cannabidiol; CBDA: Cannabidiolic acid; CBNA: Cannabinolic acid; CBN: Cannabinol; U-HPLC: Ultra-high performance liquid chromatography; MC: Medical cannabis; CBGA: Cannabigerolic acid; CBG: Cannabigerol; CBN: Cannabinol; COOH: Carboxyl group; RH: Relative humidity; VC: Vapor chips; RT: Room temperature; CI: Confidence interval; CFU: Colony forming units; Mcg: Microgram; BL: Baseline conditions.

Acknowledgements

Not applicable

Authors' contributions

Conceptualization, J.A., D. A, A. K, and I.Z.; data curation, J.A., A.K., and I.Z.; formal analysis, J.A. and D.A.; investigation, J.A. and D.A.; methodology, J.A., D.A., A.F., and A.H.; project administration, D.A. and A.H.; validation, J.A. and D.A.; visualization, J.A. and A.F.; writing—original draft, J.A.; writing—review and editing, J.A., D.A., A.F., A.K., I.Z., and A.H. All authors participated in data collection, discussed the results, and commented on the manuscript. The authors read and approved the final manuscript.

Funding

All analyses were funded by Syqe Medical Ltd.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the intellectual property of Syqe Medical, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable. No human or animal data was utilized during this study.

Consent for publication

All authors have consented to publication of this manuscript. No individual personal/private data is incorporated in this report.

Competing interests

Joshua Aviram, Daniella Atzmony, Asaf Kroll, Anna Frenklakh, and Ilana Zaks were employed by Syqe Medical Ltd. at the time the paper was written. Arno Hazekamp was an external hired consultant to Syqe Medical Ltd. at the time the paper was written. Syqe Medical Ltd. collected samples and provided them to an external laboratory as required by local regulations for independent analyses. The authors then drafted this document based on the independent laboratory's reports. Joshua Aviram, the Clinical Research Director in Syqe Medical Ltd., performed the statistical analysis of the data, prepared the first draft of the manuscript, and made the decision to publish. All authors reviewed the final version of the manuscript.

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Received: 8 February 2022 Accepted: 12 October 2022
Published online: 24 October 2022

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