CLIP Study
Statistical Analysis Plan

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1. Abbreviations

AE	Adverse Event
AR	Adverse Reaction
AUC_t	Area under the plasma concentration-time curve from time zero to time t.
AUC_{inf}	Area under the plasma concentration-time curve from time zero to time infinity.
Bioavailability	The proportion of drug absorbed into the systemic circulation
BP	Blood Pressure
C_{max}	The maximum serum concentration that a drug achieves
C_{mean}	The mean serum concentration that a drug achieves over a specified period of time
CBD	Cannabidiol
CRF	Clinical Research Facility
DEQ-5	Drug Effects Questionnaire
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GSRS	Gastrointestinal Symptom Rating Scale
HR	Heart Rate
King's CRF	NIHR Wellcome Kings Clinical Research Facility
KHPCTO	The King's Health Partners Clinical Trials Office
MHRA	Medicines and Healthcare products Regulatory Agency
NA	Not Applicable
PK	Pharmacokinetic
REC	Research Ethics Committee
RR	Respiratory Rate
PI	Principal Investigator
PPS	Per Protocol Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Drug Reactions
SLaM	South London and Maudsley NHS Foundation Trust
SUSAR	Suspected Unexpected Serious Adverse Reactions
Temp	Temperature
T_{max}	The time after administration of a drug that the C_{max} is observed
$T_{½}$	The time taken for the plasma concentration to fall by half its original value
UAR	Unexpected Adverse Reactions

2. Source Documents

The Statistical Analysis Plan (SAP) was written based on the following documentation:

Document	Date	Version
Protocol	1.2	07/MAR/2022
CRF	2.0	26/APR/2022

3. Trial Design

Single-centre double-blind two-period crossover pharmacokinetic study.

The study includes one baseline screening visit followed by two experimental visits each lasting 48 hours, separated by a minimum 2 weeks washout. Participants will return home each day and will not stay in the hospital overnight.

4. Primary objective

To assess whether the novel formulation is able to increase the bioavailability of oral CBD in the fasting state.

Hypothesis: the novel formulation will increase the AUC_{inf} for a single dose of oral CBD in the fasting state.

5. Primary outcome

Difference in AUC_{inf} for a single dose of oral CBD between the novel and standard formulations in the fasting state.

6. Secondary objectives

To characterise and compare the pharmacokinetic profiles of the novel CBD formulation and a standard formulation

7. Secondary outcomes

Differences between novel and standard formulations for:

i. Maximum plasma concentration (C_{max})
ii. Time after administration of drug when maximum plasma concentration is reached (T_{max})
iii. Plasma half-life ($t½$)
iv. Area under the concentration-time curve from time zero to 48hours (AUC_{0-48})

8. Trial assessments

8.1. Blood sampling

Blood samples will be drawn at the following time-points on experimental visits:
Pre-dose (0-5mins), 0.5, 1, 2, 3, 4, 5, 6, 8, 24, and 48hrs post-dose. The 24hr and 48hour samples should be taken within 15minutes of the target time.

8.2. Vital signs

Vital signs (HR, RR, BP, Temp) will be recorded on experimental visits at the following times: pre-dose and 1, 2 4, 8, 24 and 48hrs post-dose. Urinalysis will be completed 4hrs post-dose. The 24hr and 48hour readings should be taken within 15minutes of the target time.

8.3. Adverse events

Participants will be asked to report adverse events at each blood sampling timepoint i.e. pre-dose (0-5mins), 0.5, 1, 2, 3, 4, 5, 6, 8, 24, 48hrs post-dose and at the 7-14 day follow-up visit . Participants will be asked specifically about adverse events including, but not limited to, anxiety, irritability, somnolence, dizziness, disorientation, nausea, abdominal pain, abdominal distension, diarrhoea, vomiting, and flatulence.

8.4. Drug Effects Questionnaire (DEQ-5)
The DEQ-5 is questionnaire with five items, for example ‘‘Do you feel a drug effect, right now?’ with the response anchors ‘Not at all’ and ‘Extremely’ (Morean et al. 2013). It will be recorded on experimental visits at the following times: pre dose and at 1, 2, 4, 8, 24 and 48hrs post-dose.

8.5. Gastrointestinal Symptom Rating Scale (GSRS)
The GSRS is a 15-item rating scale which assesses five symptom clusters: reflux, abdominal pain, indigestion, diarrhoea and constipation. Each item is assessed with a 7-point Likert scale. The standard recall period for the scale is 7 days; this will be amended so that it assesses past 24 hours only. The scale will be used pre-dose and at 24 and 48 hours post dose.

8.6. Stool Sample / Gut Microbiome (Exploratory analysis)
To assess the effect of the gut microbiome on the CBD’s pharmacokinetics and circulating endocannabinoid levels, a stool sample will be collected prior to each drug administration. Participants will be provided with stool sampling kits at the screening visit. This part of the study is optional.

9. Laboratory Analyses
Blood samples will be analysed for CBD and its metabolites (including 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD) CBDA, THC, endocannabinoids (including anandamide and 2-arachidonoylglycerol). Additional unplanned analyses of relevant metabolites, biomarkers or inflammatory markers may also be completed. The laboratory analyses will be completed at the University of Turku, Finland. Analyses will be completed once all samples have been collected, at the end of the study. All analyses will be completed in accordance with relevant guidance and legislation.

10. Statistics and Data Analysis

10.1. Power analysis
As the means are unknown, we base the power calculation on minimum detectable difference assuming the AUC coefficient of variation in CBD is 50% (Taylor et al. 2018). Since there is no carry-over effect in the study, an appropriate analysis of continuous data from a two-period, two-intervention cross-over trial is a paired t-test. For the primary outcome, the bioavailability of lipid versus standard formulation in the fasted state, a paired t-test with n=12 has 80% power, at alpha=0.05 (2 tailed) to detect a 44.5% difference in means.

Previous studies demonstrated increased bioavailability with lipid formulations of 7x. The study is adequately powered to demonstrate these differences. This sample size and design satisfies EMA recommendations for design and minimum sample size (n=12) in pharmacokinetic studies for comparing formulations.

10.2. Planned recruitment
The study requires 12 complete datasets. With an expected drop-out rate of 15%, we expect to recruit 14 participants.

10.3. Analysis populations

10.3.1. Full Analysis Set (FAS)
The FAS will consist of all randomized/assigned participants who had at least one dose of treatment. FAS participants are analysed according to their randomized/assigned treatment.

10.3.2. Per Protocol Population Set (PPS) or PK analysis population

The PPS will consist of all participants with completed two experimental visits and have a measurable level for all timepoints.

10.4. Data handling

10.4.1. Visit windows

The visits are outlined as below table.

Visit	Target Day of Visit	Acceptable visit window
Experimental visit 1		
Screening visit	Day -28 to 0	NA
Day 1 visit	Day 1	NA
Day 2 visit	Day 2	NA
Day 3 visit	Day 3	NA
Washout period 2 weeks		
Experimental visit 2		
Day 1 visit	Day 1	NA
Day 2 visit	Day 2	NA
Day 3 visit	Day 3	NA
Follow up visit	Day 7-14	NA

10.4.2. PK time points windows

The target sampling days and times with windows for PK samples in Experimental visit 1 and 2 are shown in below table. In the event of repeat values within a window, the last non-missing value per study day/time will be used.

Target Day of Visit	Timepoints with window
Day 1	Pre-dose (0-5mins) 0.5-hour (post dose) +/- 5 mins 1-hour (post dose) +/- 5 mins 2-hours (post dose) +/- 5 mins 3-hours (post dose) +/- 5 mins 4-hours (post dose) +/- 5 mins 5-hours (post dose) +/- 5 mins 6-hours (post dose) +/- 5 mins 8-hours (post dose) +/- 5 mins
Day 2	24-hours (post dose) +/- 5 mins
Day 3	48-hours (post dose) +/- 5 mins

10.4.3. Handling of Dropouts, Missing Data, and Outliers

There are no imputations planed for missing data.

Partial dates and missing dates will not be imputed. Any classifications based on partial or missing dates will assume a “worse case” scenario. If a medication cannot be classified as either prior or concomitant due to missing or partial start and stop dates, it will be considered to be concomitant. If an AE cannot be classified as resolved prior to the end of the study period due to missing or partial stop dates, it will be assumed to be ongoing.

10.5. Statistical Methods

10.5.1. General Principles

All data processing, summarization and analyses will be performed using Greenlight Clinical's SAS Environment / Version 9.4 (or later) of the SAS® statistical software package.
The following principles will be applied to all tables, listing and figures unless otherwise stated in below table.

Principle	Value
Significant tests	Two-sided and use a 5% significance level for main effects and 10% significance level for interaction terms.
Treatment group labels and order presented	SEEK-CBD STD-CBD
Tables	Data in summary tables presented by treatment formulation and visit (where applicable).
Listings	All data collected presented by treatment formulation, subject number and visit (where applicable), unless otherwise specified.
Descriptive summary statistics for continuous variables	Number of subjects/observations (N), mean, standard deviation (SD), median, minimum(min) and maximum(max)
Descriptive summary statistics for categorical variables	Frequency counts and percentages [n (%)]
Denominator for percentages	Number of subjects in the analysis population, unless stated otherwise in table shell(s)
Include "Missing" as category	No, N will change, unless stated otherwise
Display for 0 percentages	-
Display to one more decimal place than collected value	Mean Standard Error Standard Deviation Mean Difference Median Minimum Maximum
Display to two more decimal places than collected value	Confidence Interval
Limit of precision for displays	3 decimal places
Date Format	YYYY-MM-DD

10.5.2. Subject Disposition and Data Sets Analysed

Subject disposition will be listed and summarized by treatment group and will include the number and percentage of subjects:

- Screening
- Randomized
- Randomized and not treated
- Randomized and treated
- Included in the FAS
- Included in the PK analysis population
- Complete study

In addition, the number and percentage of subjects who complete the study and who discontinue early, including a breakdown of the primary reasons for discontinuation, will be presented for all analysis populations.

Subject disposition will be presented in a listing and the reason for discontinuation.

10.6. Statistical analysis plan

10.6.1. Protocol violations

All protocol violations, including inclusion/exclusion criteria violations and violations during the trial, will be listed, even if they are believed not to influence any of the results.

10.6.2. Demographics

For all participants, descriptive statistics of demographic (e.g., sex, age, BMI, and other baseline characteristics) will be presented by overall.

10.6.3. Safety measures

Summary tables showing vital signs e.g., body temperature, respiratory rate, pulse rate, and SBP and DBP will be provided at each study time point and changes from baseline.
Individual physical examination will be listed and summarised at each study time point.
Important Medical Event, drug, pregnancy test results during the study will be listed.

10.6.4. Visual Analogue Scales & Drug Effects Questionnaire (DEQ-5)

A summary table of DEQ-5 will be summarised descriptively.

10.6.5. Laboratory results

Laboratory parameters including haematology, blood chemistry, and urinalysis will be listed accompanied by an indication if the parameter is outside the reference range. A summary of all data outside the reference range of the clinical laboratory will be provided. Clinical laboratory data will be presented descriptively, where applicable.

10.6.6. Pharmacokinetics parameters

Pharmacokinetic parameters will be summarized descriptively. Continuous outcomes will be reported as means with standard deviation. Categorical outcomes will be reported as frequencies. Individual participant's pharmacokinetic data may also be presented in tables and graphs. The plasma concentration-time data will be subject to non-compartmental pharmacokinetic analysis using appropriate computer software applications. Linear and/or log-linear plots will be presented. To assess bioavailability between the formulations, log transformed AUC, C_{max} observations will be entered into a linear mixed model to account for the repeated measures and between subject conditions. Linear contrasts representing the difference between conditions will be expressed as ratio of geometric means along with 95% CI and inference based on $p < 0.05$. Planned analysis will be per protocol.

10.6.7. Adverse events

A listing of all individual AEs, AR, UAR, SAE, SAR, and SUSAR will be provided. Summary tables of TEAEs will be presented by system organ class based on the MedDRA terminology list (preferred terms): 1 containing the number of TEAEs (frequency of occurrence, number of participants/patients experiencing the event) by treatment and 1 containing the number of drug related TEAEs (frequency of occurrence, number of participants/patients experiencing the event) per treatment. Additional tables of total counts by treatment and relationship and by treatment and severity will be given.

The final statistical analysis plan will be in place prior to study database lock.