soundBrilliance™

Statistical Analysis Plan

Impacts of Music, Audio Enhancements, Audio Guidance, and Visuals on Physical and Emotional Well-being. (Emotional Health - Longitudinal 1 Randomized, Controlled Multi-center Study)

A 12-Week Parallel-Group Randomized Controlled Trial

Field	Details
NCT Number	[Not yet assigned]
Unique Protocol ID	SB EMH L 001
Protocol Version	1.0 / Aprill 22, 2026
SAP Version & Date	1.0 / April 22, 2026
Sponsor	SoundBrilliance
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Programming Language	R (version $\geq$ 4.3.0)

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List of Abbreviations

Abbreviation	Description
CI	Confidence Interval
CS	Compound Symmetry
CSH	Heterogeneous Compound Symmetry
CSR	Clinical Study Report
EMM	Expected Marginal Mean
FWER	Family-wise error rate
ICH	International Council for Harmonization
ITT	Intent-to-Treat
KR	Kenwood-Roger
MAAS	Mindful Attention Awareness Scale
MAIA	Multidimensional Assessment of Interoceptive Awareness
mITT	Modified Intent-to-Treat
MAR	Missing at Random
MI	Multiple Imputation
ML	Maximum Likelihood
MMRM	Mixed Model for Repeated Measures
PANAS-AF	Positive and Negative Affect Schedule Short Form
PHQ-8	Patient Health Questionnaire
PNS	Parasympathetic Nervous System
PP	Per-Protocol
PSS	Perceived Stress Scale
RCT	Randomized Clinical Trials
REML	Restricted Maximum Likelihood
RMSSD	Root Mean Square of Successive Differences
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error
SI	Stress Index
SNS	Sympathetic Nervous System
STAI	State-Trait Anxiety Inventory
TEAE	Treatment Emergent Adverse Event
TOEPH	Heterogeneous Toeplitz
UN	Unstructured

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1 Introduction

1.1 Purpose

This Statistical Analysis Plan (SAP) pre-specifies all statistical methods to be applied in the analysis of data from this 12-week parallel-group randomized controlled trial (RCT). The SAP is intended to minimize analysis bias by defining, prior to database lock, all primary, secondary, and exploratory analyses, as well as data handling rules and model specifications.

This document should be read in conjunction with the study protocol. In the event of any discrepancy, the SAP takes precedence for statistical decisions.

1.2 Study Overview

Parameter	Description
Design	Parallel-group, randomized, controlled trial
Duration	12 weeks
Treatment Arms	[Active Treatment] vs. [Control/Placebo]
Randomization	1:1?
Assessment Timepoints	Weeks 0, 2, 4, 6, 8, 10, 12
Primary Endpoint	[Specify primary outcome measure]
Target Sample Size	100 per arm (200 total)
Primary Analysis Model	Linear Mixed-Effects Model (MMRM)

2 Study Objectives and Endpoints

2.1 Primary Objective

To evaluate the overall effect of [treatment] versus [control] on *State Anxiety* (STAI-S), *Depression* (PHQ-8), and *Affect* (PANAS-SF) over 12 weeks.

2.2 Primary Endpoint

State Anxiety, *Depression*, and *Affect* assessed across the 12-week study period. The primary estimand is the difference in the overall change from baseline across the 12-week study period between treatment arms under the intent-to-treat (ITT) estimand framework (ICH E9(R1)).

2.3 Key Secondary Endpoints

- Perceived Stress (PSS) will be measured at baseline and at the end of the 12-week study period.
- Quality of Life (Cantril's Ladder) will be measured at baseline and at the end of the 12-week study period.

Differences in the change from baseline will be compared between the treatment arms.

2.3.1 Secondary Endpoints

A subset of participants will receive commercial-grade ECG equipment (Polar Pacer watch/H10 chest strap) and will be asked to wear the equipment when completing in-app sessions. Secondary dependent variables measured with this equipment include

- root mean square of successive differences (RMSSD)

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- parasympathetic nervous system (PNS) activation,
- sympathetic nervous system (SNS) activation, and
- stress index (SI).

2.4 Exploratory Endpoints

- Subgroup analyses by demographic (age/sex) and clinical characteristics (baseline Trait-Anxiety Level).

3 Analysis Populations

Population	Definition	Primary Use
Intent-to-Treat (ITT)	All randomized subjects, analyzed in assigned arm regardless of compliance	Primary analyses
Modified ITT (mITT) & Randomized subjects who received ≥ 1 dose and have ≥ 1 post-baseline assessment		Sensitivity analyses
Per-Protocol (PP)	mITT subjects with no major protocol deviations, defined prior to unblinding	Sensitivity analyses
Safety Population	All subjects who received ≥ 1 dose of study treatment	Safety analyses

The ITT population is the primary analysis population. All randomization stratification factors will be included as covariates in the primary model regardless of population used.

4 General Statistical Methods

4.1 Significance Level and Multiplicity

A two-sided significance level of $\alpha = 0.05$ will be used for the primary endpoint. All confidence intervals (CIs) will be 95% two-sided. For secondary endpoints, a hierarchical testing procedure will be applied to control the family-wise error rate (FWER); results for secondary endpoints will only be interpreted as confirmatory if the primary endpoint achieves statistical significance.

No multiplicity adjustment is applied to exploratory endpoints; these will be interpreted as hypothesis-generating only.

4.2 Descriptive Statistics

Continuous variables will be summarized with: n, mean, standard deviation (SD), median, 25th and 75th percentiles, minimum, and maximum. Categorical variables will be summarized with frequency counts and percentages. All summaries will be presented by treatment arm and overall.

4.3 Handling of Covariates

Randomization stratification factors will be included in all primary and secondary inferential models as fixed effects, regardless of observed balance. Additional baseline characteristics identified a priori in the protocol will also be included where specified.

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4.4 Software

All analyses will be conducted in R (version $\geq$ 4.3.0). The primary mixed model will be fitted using the `nlme` and/or `lme4` packages. Key packages include:

```
nlme         # lme(), primary MMRM implementation
lme4         # lmer(), alternative/sensitivity implementation
emmeans      # Estimated marginal means and contrasts
broom.mixed  # Tidy model output
ggplot2      # Figures
mice         # Multiple imputation (sensitivity)
```

R session information (`sessionInfo()`) will be saved and archived as part of the analysis outputs. A random seed of [specify] will be set at the start of each analysis script for reproducibility.

5 Missing Data

5.1 Missing Data Assumptions

The primary analysis assumes data are Missing At Random (MAR), conditional on observed covariates and prior measurements included in the mixed model. This assumption is plausible because the MMRM uses all available observed data without explicit imputation.

5.2 Primary Approach

The MMRM uses all available observed longitudinal data via maximum likelihood (ML) or restricted maximum likelihood (REML) estimation. Subjects with any missing post-baseline assessments are retained in the model and contribute observed data to all timepoints at which they were assessed.

5.3 Sensitivity Analyses for Missing Data

- **Multiple Imputation (MI):** Missing values will be imputed under MAR using predictive mean matching (`mice` package, m $\geq$ 20 imputed datasets). The MMRM will be re-fitted on each imputed dataset and results combined via Rubin's rules.

6 Primary Analysis

6.1 Model Specification

The primary analysis will use a Mixed Model for Repeated Measures (MMRM). The model is specified as:

$$\begin{aligned} Y_{ij} = \beta_0 + \beta_1 Treatment_i + \beta_2 Time_j + \beta_3 Baseline_i + \beta_k Strata_k + \\ \beta_5 (Treatment_i \times Time_j) + \beta_l (Treatment_i \times Strata_k) + \beta_m (Time_j \times Strata_k) + \\ \beta_n (Treatment_i \times Strata_k \times Time_j) + b_i + \varepsilon_{ij} \end{aligned}$$

Term	Type	Description
Y_{ij}	Outcome	Primary outcome for subject i at time j
β_1(Treatment)	Fixed	Main effect of treatment arm (Active vs. Control)

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Term	Type	Description
β_2(Time)	Fixed	Main effect of time (Weeks 2, 4, 6, 8, 10, 12; categorical)
β_3(Baseline)	Fixed	Baseline value of primary outcome (continuous covariate)
β_k(Strata)	Fixed	Randomization stratification factor(s)
β_5(Treatment $\times$ Time)	Fixed	Treatment-by-time interaction
β_l(Treatment $\times$ Strata)	Fixed	Treatment-by-strata interaction
β_m(Time $\times$ Strata)	Fixed	Time-by-strata interaction
β_m(Treatment x Strata x Time)	Fixed	Treatment-by-Strata-by-Time interaction
$b_i \sim N(0, \sigma_b^2)$	Random	Subject-level random intercept
$\varepsilon_{ij} \sim N(0, \Sigma)$	Residual	Within-subject errors with unstructured covariance

6.2 Covariance Structure

An unstructured (UN) residual covariance matrix across timepoints will be used as the primary specification, as it makes no assumptions about the pattern of within-subject correlation. If the UN model fails to converge, the following structures will be considered in order:

- Heterogeneous Toeplitz (TOEPH)
- Heterogeneous Compound Symmetry (CSH)
- Compound Symmetry (CS)

The selected structure will be documented and the rationale stated.

6.3 Estimation and Inference

Model parameters will be estimated using Restricted Maximum Likelihood (REML). The primary estimand is the estimated overall marginal mean (EMM) difference between treatment arms across the 12 weeks, derived from the Treatment parameters using the `emmeans` package. Degrees of freedom will be approximated using the Kenward-Roger (KR) method to ensure valid inference in small samples.

6.4 R Implementation

```r
library(nlme)
library(emmeans)

# Ensure time is a factor (categorical)
dat$week <- factor(dat$week, levels = c(0, 2, 4, 6, 8, 10, 12))
dat$trt  <- factor(dat$trt, levels = c("Control", "Active"))

# Fit primary MMRM with unstructured residual covariance
fit_primary <- lme(
  outcome ~ trt * week * strata + baseline,
  random  = ~ 1 | subject_id,
  data    = dat,
  na.action = na.omit,
  weights = varIdent(form = ~ 1 | week),
```

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```r
  method  = "REML"
)

# Estimated overall marginal means and contrast
emm <- emmeans(fit_primary, ~ trt, mode = "kenward-roger")
contrast(emm, method = "pairwise", simple = "trt")
```

7 Secondary Analyses

7.1 Longitudinal Trajectories (All Timepoints)

The same MMRM specified in Section 6 will be used to estimate treatment differences at each individual timepoint (Weeks 2, 4, 6, 8, 10, and 12). EMMs, contrasts, and 95% CIs will be reported at each timepoint.

7.2 Subgroup Analyses

EMMs, contrasts, and 95% CIs for subgroups and subgroup interactions will also be estimated from the model described in Section 6.

7.2.1 Single-Arm Analyses

For the measurements described in section 2.3.1, shift tables will be created to reflect the categorical change from baseline. These data will be analyzed using Cochran's Q test (or McNemar's test if only two categories).

8 Sensitivity Analyses

Sensitivity Analysis	Purpose	Method
Per-Protocol population	Exclude protocol deviators	Re-run primary MMRM on PP population
Alternative covariance structure	Assess covariance model dependency	CS, CSH, AR(1) structures
Multiple imputation under MAR	Assess MAR assumption	mice (m=20), REML per imputed dataset, Rubin's rules
Alternative random effects	Random slope model	Add random slope for time: `(1 + week | subject_id)`

9 Subgroup Analyses

Pre-specified subgroup analyses will be conducted for the primary endpoint. These are considered exploratory and are not adjusted for multiplicity. A treatment-by-subgroup-by-time interaction test will be performed.

Subgroup Variable	Categories (Levels)
Age	18-49, 30-49, 50+ years
Sex	Male vs. Female
Baseline Trait-Anxiety Category (STAI-2)	No or Low, Moderate, High (per protocol definition)
Baseline Perceived Stress Category (PSS)	Low, Moderate, High (per protocol definition)
[Stratification factor k]	[Levels]

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Results will be presented in a forest plot showing treatment differences and 95% CIs within each subgroup.

10 Safety Analyses

Safety analyses will be performed on the Safety Population (all subjects receiving ≥ 1 dose). No formal hypothesis testing will be conducted on safety data; all summaries are descriptive.

10.1 Adverse Events

- will include: number of subjects with ≥ 1 event, event counts, and percentages by arm
- Serious adverse events (SAEs) and events leading to discontinuation will be listed individually

11 Tables, Listings, and Figures

Output	Content	Population
Table 1	Subject disposition	All randomized
Table 2	Baseline demographics and clinical characteristics	ITT
Table 3	Primary endpoint: MMRM results across Week 12	ITT
Table 4	Primary endpoint: EMMs at all timepoints	ITT
Table 5	Subgroup analysis forest plot data	ITT
Table 6	TEAE summary	Safety
Table 7	SAE listing	Safety
Figure 1	Mean (±SE) trajectory over time by arm	ITT
Figure 2	Forest plot of subgroup treatment effects	ITT
Listing 1	Subject-level primary outcome data	ITT

12 Model Diagnostics

The following diagnostics will be performed and documented for the primary MMRM:

- **Residual Plots:** Pearson residuals vs. fitted values, Q-Q plots of normalized residuals
- **Random effects distribution:** Q-Q plot and histogram of estimated subject random intercepts
- **Influence diagnostics:** Cook's distance and leverage for outlier identification
- **Convergence:** Gradient norm and log-likelihood at convergence to be reported
- **Variance component estimates:** σ_b^2 (between-subject variance) and residual variance matrix Σ to be tabulated

```
# Residual diagnostics
plot(fit_primary)                          # Residuals vs fitted
qqnorm(resid(fit_primary, type = "normalized"))
qqline(resid(fit_primary, type = "normalized"))

# Random effects
ranef_vals <- ranef(fit_primary)$subject_id
qqnorm(ranef_vals[[1]]); qqline(ranef_vals[[1]])
```

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13 Randomization and Blinding

Randomization will be performed using [block randomization / minimization] with stratification by *[specify factors]*. The randomization list will be generated by statistician prior to study start.

The SAP will be finalized and time-stamped prior to unblinding. Any post-hoc deviations from the SAP will be documented in the clinical study report (CSR) with full justification.

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14 SAP Version Control and Amendments

Version	Date	Author	Description of Changes
1.0	2026-04-17	Timothy W. Victor, Ph.D.	Initial version

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15 Approval Signatures

Role	Name	Signature	Date
Principal Investigator	Jason S. Doescher, M.D.		
Lead Statistician	Timothy W. Victor, Ph.D.		

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16 References

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