STATISTICAL ANALYSIS PLAN

A TWO-PERIOD, TWO-SEQUENCE, TWO-TREATMENT, SINGLE-DOSE CROSSOVER STUDY OF ATROPINE SULFATE OPTHALMIC SOLUATION (1%) ADMINISTERED SUBLINGUALLY VERSUS ATROPINE SULFATE ADMINISTERED INTRAMUSCULARLY FOR BIOEQUIVALENCE DETERMINATION

BP-C-24-001

SHORT TITLE:	Sublingual Atropine Bioequivalence by Route of Administration (SABER)
NCT#:	NCT06366087
COMPOUND #:	NDC 82260-0001-01 (Atropine Sulfate Ophthalmic Solution, USP 1%)
CLIENT:	BARDA
REGULATORY AGENCY IDENTIFIER NUMBER(S):	N/A
PREPARED BY:	Rho [redacted] Telephone: [redacted] Fax: [redacted]

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ACKNOWLEDGEMENT AND SIGNATURE SHEET

Rho Project Statistician Approval	Rho Principal Investigator Approval
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Document History

Version	Date	Change(s)	Author
1.0	25 APR 2024	Initial Version	[redacted]

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1. LIST OF ABBREVIATIONS

Table 1: Abbreviations

Abbreviation	Explanation
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
AUC_{∞}	Area under the analyte concentration versus time curve to infinity
AUC_t	Area under the analyte concentration versus time curve to time of last quantifiable data point
BARDA	Biomedical Advanced Research and Development Authority
BMI	Body Mass Index
CI	Confidence Interval
CL/F	Apparent total body clearance after extravascular administration
C_{max}	Maximum measured plasma concentration
CV%	Coefficient of Variation
CRF	Case Report Form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DSMP	Data Safety Monitoring Plan
ECG	Electrocardiogram
EDC	Electronic Data Capture
ICH	International Council for Harmonisation
ID	Identification Number
IM	Intramuscular
K_a	Absorption rate constant
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NCI	National Cancer Institute
PK	Pharmacokinetic
PT	Preferred Term
SABER	Sublingual Atropine Bioequivalence by Route of Administration

Abbreviation	Explanation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
SL	Sublingual
SOC	System Organ Class
SVP	Statistical Validation and Quality Control Plan
$t_{1/2}$	Apparent terminal elimination half-life
t_{max}	Time to maximum measured plasma concentration
TEAE	Treatment Emergent Adverse Event
V_d/F	Apparent total volume of distribution after extravascular administration
WHO	World Health Organization
λz	Terminal elimination rate constant

2. PURPOSE OF THE ANALYSES

The purpose of this Statistical Analysis Plan (SAP) is to describe the planned analyses and data displays to be included in the Clinical Study Report (CSR) for Protocol BP-C-24-001/SABER. This document provides details on study populations, how the variables will be derived, how missing data will be handled and details on statistical methods to be used to analyze the safety and pharmacokinetic data.

The SAP is based on International Council for Harmonisation (ICH) guidelines E3, E9 (Statistical Principles for Clinical Trials), and E9 (R1)[1].

The document may evolve over time, for example, to reflect the requirements of protocol amendments or regulatory requests. However, the final SAP must be finalized, approved by Biomedical Advanced Research and Development Authority (BARDA), and placed on file before database is locked and pharmacokinetic analysis occurs. If differences occur between analyses described in the SAP and the current protocol, those found in this SAP will assume primacy. Deviations from the final approved plan will be noted in the CSR. Table, figure, and listing specifications are provided in separate documents.

3. PROTOCOL SUMMARY

Name of Sponsor/Company: Biomedical Advanced Research and Development Authority (BARDA)	
Name of Study Drug: Test Drug: Atropine Sulfate Ophthalmic Solution, USP 1% (NDC 82260-0001-01) Reference Drug: Atropine Sulfate Injection, USP 8 mg/20 mL (0.4 mg/mL) in a multidose vial presentation (NDC 63323–580-20)	
Name of Active Ingredient: Atropine sulfate	
Title of Study: A Two-period, Two-sequence, Two-treatment, Single-dose Crossover Study of Atropine Sulfate Ophthalmic Solution (1%) Administered Sublingually vs Atropine Sulfate Administered Intramuscularly for Bioequivalence Determination	
Short Title: Sublingual Atropine Bioequivalence by Route of Administration (SABER)	
Protocol Number: BP-C-24-0001	
Version Number: 1.0	
Study center(s): 1 (one)	
Study period (years): 0.5 Estimated date first participant enrolled: April 2024 Estimated date last participant completed: August 2024	**Phase of development:** Phase 1
Study Objectives: **Primary Objective:** • To establish bioequivalence of Atropine Sulfate Ophthalmic Solution, USP 1% given sublingually (SL) vs intramuscular (IM) 8 mg/20 mL (0.4 mg/mL) atropine sulfate injection. **Secondary Objective:** • To compare the bioavailability of Atropine Sulfate Ophthalmic Solution, USP 1% given SL vs IM 8 mg/20 mL (0.4 mg/mL) atropine sulfate injection.	
Methodology: This is a randomized, two-period, two-sequence, crossover study to assess the bioequivalence, bioavailability, and pharmacokinetics (PK) of a single dose of atropine administered SL or IM in healthy adult volunteers. Approximately 46 healthy male and non-pregnant female volunteers will be enrolled to obtain approximately 36 evaluable participants in the per protocol population. Additional participants may be randomized if participants are withdrawn prior to receiving one or both doses of study drug. Eligible participants will be randomized at a 1:1 ratio to receive one of two treatment dosing sequences (A or B) as depicted in the table below: **Study Design Scheme by Dosing Sequence**	

Dosing Sequence	Targeted Number of Randomized Participants (N)	Targeted Number of Evaluable Participants (N)	Period 1 (Visit 1; Day 1)	Period 2 (Visit 2; Day 8)
A	23	18	SL	IM
B	23	18	IM	SL

Volunteers will be screened for study participation from Days -14 to -3. After Screening on Day 1, eligible participants will be enrolled and will be randomized to receive one of two dosing sequences as presented in the table above. Once randomized, each participant will receive 2 doses of atropine separated by a wash out period of 6 ±1 days. During Visit 1 (Day 1) and Visit 2 (Day 8), participants will be administered SL or IM atropine, according to their randomly assigned dosing sequence. At each dosing visit, blood samples for PK will be collected at time 0 (pre-dose) and at 13 time points post-dose at 5, 10, 15, 20, 30, 45, 60, and 90 minutes and 2, 2.5, 4, 6, and 8 hours after dosing. Participants will be discharged from the clinic after the 8-hour blood sample collection. Participants will be followed for approximately 6 days after their last dose.

Number of participants (planned): Approximately 46 enrolled for 36 evaluable

Diagnosis and main criteria for inclusion: Healthy male and non-pregnant female participants aged 18 through 65 years, inclusive.

Eligibility criteria will be assessed at Screening and must be reviewed just prior to each dose of atropine. Criteria specified as only being evaluated at Screening or at Pre-dose Visits will be assessed only at those visits. If the participant no longer meets applicable eligibility criteria, the investigator, in consultation with the medical monitor in cases of uncertainty, must determine whether the participant should receive the atropine dose or be terminated early from study drug.

Participant Inclusion Criteria

1. Healthy male and non-pregnant female volunteers between the ages of 18 and 65 years, inclusive, at time of consent.
2. Willing and able to provide written informed consent.
3. Females who are of childbearing potential and are sexually active with a male partner must have used an adequate method of birth control for at least 2 months prior to Screening and must agree to continue using an adequate method of birth control from Screening through Follow-up (Day 15).
    a. A female of childbearing potential is defined as a post onset of menarche and premenopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: menopausal >2 years, tubal ligation >1 year, bilateral salpingo-oophorectomy, or hysterectomy.

b. Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label. Examples include oral contraceptives, injectable progestogen, implants of etonogestrel or levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, intrauterine device or intrauterine system, or male partner sterilization at least 6 months prior to the participant's Screening Visit.

4. In the judgment of the investigator, the participant is in good health, based on review of medical history and the results of Screening evaluation (including vital signs, physical examination, 12-lead electrocardiogram [ECG], and Screening laboratory assessments, performed no more than 14 days prior to randomization into the study).
5. Able to comply with the dosing instructions and available to complete the study Schedule of Assessments.

Participant Exclusion Criteria

1. Females who have a positive pregnancy test or who are breastfeeding.
2. Participants with thyroid disease as evidenced by a thyroid-stimulating hormone (TSH) <0.9 × lower limit of normal (LLN) or > 1.2 × upper limit of normal (ULN) at Screening (This Screening test will not be repeated prior to subsequent dosing).
3. Participants with aspartate aminotransferase (AST), alanine aminotransferase (ALT), or serum creatinine >1.5 × ULN at Screening. (These Screening tests will not be repeated prior to subsequent dosing.)
4. Have known human immunodeficiency virus (HIV), or acute or chronic hepatitis B or hepatitis C infection based on medical history; or test positive for any of these at Screening. Participants who have been effectively treated for hepatitis C, as evidenced by a negative hepatitis C RNA confirmation test and who no longer require antiviral therapy, are eligible for participation. (These Screening tests will not be repeated prior to subsequent dosing.)
5. Participants who took any prescription medications (with the exception of oral contraceptives or hormone replacement therapy) within 30 days of Screening. Prior to each dose, the investigator will review prohibited medication use and determine whether the participant should be terminated from further dosing.
6. Participants who took any over-the-counter medication/vitamins/herbal supplements in the last 72 hours prior to Screening. Prior to each dose, the investigator will review prohibited medication use and determine whether the participant should be terminated from further dosing.
7. Participants who are current smokers or are currently using any oral nicotine/oral tobacco product (e.g. snuff, chew, lozenges, nicotine gum, pouches) or electronic cigarette or vaping device (e.g., e-cigarette, mod, vape pen, JUUL, e-cigar, e-hookah, e-pipe, vape pods) or have used any of these products within 6 months prior to Screening.

8. Participants with glaucoma and/or history of ocular surgery (including Lasik™), ocular trauma, or congenital ocular disorder.
9. Participants with any history of heart disease, including but not limited to hypertension, coronary artery disease, arrhythmia (treated or untreated), congestive heart failure, pacemaker, history of vasovagal syncope, any supraventricular tachycardia, peripheral vascular disease, or claudication.
10. Participants with clinically significant arrhythmias or abnormal conduction; abnormal conduction is defined as a prolonged PR or QRS, or a QTc ≥450 msec for males or ≥470 msec for females.
11. Participants with a history of partial organic pyloric stenosis, chronic constipation, gastroparesis, or other gastrointestinal motility issues.
12. Participants with a history of xerostomia due to an underlying disease or previous radiation therapy to the head and neck.
13. Males with history of symptomatic prostatic hypertrophy; males or females with a history of urinary hesitancy or retention.
14. Participants with a blood pressure >140/90 mm Hg taken after the participant has been seated and resting for at least five minutes.
15. Participants with a history or current diagnosis of myasthenia gravis.
16. Participants who have donated blood within 8 weeks of Screening or intend to donate blood during the study period.
17. Participants with a history of drug or alcohol abuse in the last two years or evidence of a positive urine drug test at Screening. (This Screening test will not be repeated prior to subsequent dosing.)
18. Participants with a known sensitivity or prior adverse reaction to atropine.
19. Participants who have consumed alcohol within 24 hours prior to each Pre-dose Visit. (This will be assessed only at Visit 1 [Pre-dose] and Visit 2 [Pre-dose].)
20. Participants with any other condition that, in the opinion of the investigator, would pose a health risk to the participant if enrolled, would interfere with SL or IM administration of study drug, or would otherwise interfere with interpretation of study results.

Participants cannot be rescreened for exclusionary laboratory test results. Potentially exclusionary vital sign results may be repeated once. If a participant's repeat vitals remain exclusionary or the investigator determines that the repeat vital signs could pose a risk to participating in the study, then the participant will be excluded.

Test drug, dosage, and mode of administration: Atropine Sulfate Ophthalmic Solution USP, 1% (NDC 82260-0001-01), 1 mg (100 µL of a 1% w/v solution), administered SL by pipette with at least a 30 second dwell time without swallowing

Duration of treatment: Participants will receive two doses of atropine sulfate separated by approximately 7 days

Reference drug, dosage, and mode of administration: Atropine Sulfate Injection, USP 8 mg/20 mL (0.4 mg/mL) in a multidose vial presentation (NDC 63323–580-20), 1 mg (2.5 ml) administered by IM injection into the mid-anterolateral thigh

Criteria for evaluation: Pharmacokinetic parameters only (bioequivalence/bioavailability)

Study Endpoints

Primary Endpoints

The bioequivalence of atropine sulfate administered SL versus administered IM as measured by the following PK parameters:

- Area under the analyte concentration versus time curve to infinity (AUC_{∞})
- Area under the analyte concentration versus time curve to time of last quantifiable data point (AUC_t)

Secondary Endpoints

The relative bioavailability of atropine sulfate administered SL versus IM as measured by the following PK parameters:

- Area under the analyte concentration versus time curve to time 45, 60, 90, 120, 150, and 240 minutes (AUC_{45}, AUC_{60}, AUC_{90}, AUC_{120}, AUC_{150}, and AUC_{240}, respectively)
- Maximum measured plasma concentration (C_{max})
- Time to C_{max} (t_{max})
- Apparent terminal elimination half-life ($t_{1/2}$)
- Terminal elimination rate constant (λ_z)
- Volume of distribution (V_d/F)
- Total body clearance (CL/F)
- Absorption rate constant (K_a)

Statistical methods:

Analysis Plan

Statistical analyses will be performed using SAS® software Version 9.4 or later. Phoenix WinNonLin software Version 8.3 or later will be used to generate PK parameter estimates.

Descriptive statistics will be used to summarize participant characteristics and safety. These summaries will be presented overall and separately for each route of administration.

Pharmacokinetic parameter estimates will be summarized using descriptive statistics. Bioavailability of SL administration of atropine sulfate relative to that of IM atropine sulfate will be assessed based on ratios of least-square means and their associated 90% confidence intervals (CIs) generated from linear mixed models including terms for dosing sequence, route, and period as fixed effects, and subject nested within dosing sequence as a random effect. Bioequivalence will be considered met if the 90% CI of the ratio for AUC_{∞} and AUC_t lie within 80.00 to 125.00%.

Additional details of the statistical analyses, methods, and data conventions will be described in the Statistical Analysis Plan (SAP).

Final Analyses

A clinical study report will be written to include all PK and safety data collected throughout the study. For the final analysis, the study database will be monitored, cleaned, and locked per associated study plans. Further details will be specified in the SAP.

Analysis Populations

PK Analysis Population

The PK analysis population will include all participants who are randomized, receive at least 1 study drug dose, and have PK samples collected for that period. Each participant's data will be analyzed according to the corresponding route of administration for the applicable period. The PK analysis population will be used for all PK analyses.

Per Protocol Population

The per protocol population will include all participants who are randomized, receive atropine via both routes of administration according to their randomized dosing sequence, and have PK samples collected for both periods. In addition, participants with major protocol deviations may be excluded if it is determined that the deviations affect the integrity of PK data. Each participant's data will be analyzed according to the corresponding route of administration for the applicable period. Primary and secondary analyses will be repeated in the per protocol population if it differs from the PK analysis population.

Safety Population

The safety population will include all participants who are randomized and receive at least 1 study drug dose. Each participant's data will be analyzed according to the corresponding route of administration for the applicable period. The safety population will be used for all safety displays.

4. GENERAL ANALYSES AND REPORTING CONVENTIONS

The following is a list of general analysis and reporting conventions to be applied for this study:

- Validation and quality control of analysis datasets, tables, listings, and figures will be performed as specified in the Statistical Validation and Quality Control Plan (SVP).
- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form n (%). Percentages will be rounded to one decimal place. If n=0, then no percent will be shown. To ensure completeness, all summaries for categorical and discrete variables will include all reportable categories, even if none of the participants had a response in a particular category.
- Numeric variables will be summarized using n, mean, standard deviation (SD), median, minimum (min), and maximum (max). For variables summarized using geometric means, coefficient of variation (CV%) of the geometric mean will be presented in lieu of SD. The min/max will be reported at the same level of significance as original data. The mean and median will be reported at one more significant digit than the precision of the data. SD and confidence intervals (CIs) will be reported at two more significant digits than the precision of the data. Precision may be modified based on clinical judgement.
- The median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics including t and z test statistics will be reported to two decimal places.
- P-values will be reported to 3 decimal places if greater than or equal to 0.001. If less than 0.001, the value will be reported as "<0.001".
- In general, listings will be displayed by dosing sequence and participant and will be sorted in the order that columns are displayed, starting with the first column on the left.
- All analyses will be performed using the SAS System version 9.4 or higher[2]. Figures will be programmed using SAS version 9.4 or higher or R version 3.4.1 or higher.
- Dates will be displayed as ddmmmyyyy (e.g., 26May2023).
- Calculations using dates will be performed as noted below.

$$Visit\ 1\ Date = Day\ 1$$

Study day for dates after Visit 1/Day 1

$$Study\ Day = Date - Visit\ 1\ Date + 1$$

Study day for dates prior to Visit 1/Day 1

$$Study\ Day = Date - Visit\ 1\ Date$$

Total duration of days

Duration = End Date – Start Date + 1

Note: no calculation will be needed for age as it will be pulled directly from the clinical database.

- For data collected at both screening and pre-dose on Visit 1/Day 1, the baseline data will be defined as the latest non-missing value collected. Otherwise, if data are collected at only one of Screening or Visit 1/Day 1, the collected value will be considered the baseline value for analyses.

If departures from these general conventions are present in the specific evaluation section of this SAP, then those conventions will take precedence over these general conventions.

5. ANALYSIS POPULATIONS

5.1. Consented Population

This includes all participants who give written informed consent. As such, this will include participants who are and are not randomized.

5.2. Not Randomized Population

This includes all participants who give written informed consent and are not randomized. Apart from reporting the reasons for not meeting inclusion/exclusion criteria or for not being randomized, those who are not randomized will not be included in any other analyses.

5.3. Randomized Population

The randomized population will include all participants who are randomized into the study, regardless of actually receiving a study drug dose. Each participant's data will be analyzed according to the randomized dosing sequence.

5.4. Safety Population

The safety population will include all participants who receive at least 1 study drug dose. Each participant's data will be analyzed according to the corresponding route of administration for the applicable period. The safety population will be used for all safety displays.

5.5. PK Analysis Population

The PK analysis population will include all participants who are randomized, receive at least 1 study drug dose, and have PK samples collected for that period. Each participant's data will be analyzed according to the corresponding route of administration for the applicable period. The PK analysis population will be used for all PK analyses.

5.6. Per Protocol Population

The per protocol population will include all participants who are randomized, receive atropine via both routes of administration according to their randomized dosing sequence, and have PK samples collected for both periods. In addition, participants with major protocol deviations may be excluded if it is determined that the deviations affect the integrity of PK data. Each participant's data will be analyzed according to the corresponding route of administration for the applicable period. Primary and secondary analyses will be repeated in the per protocol population if it differs from the PK analysis population.

6. VALIDATION AND QUALITY CONTROL PROCEDURES

Validation and quality control of datasets, summary tables, data listings, figures, and statistical analyses will be performed in accordance with the SVP.

7. STUDY PARTICIPANTS

7.1. Disposition of Participants

The disposition of all consented participants will be tabulated and listed. The numbers and percentages of participants consented, randomized, and terminating early, including reasons for early termination, will be presented. Percentages will be based on the number of participants at each level of summarization and within the specific study group, as applicable.

In addition, the reasons for ineligibility at screening/first study drug administration and ineligibility at the time of the second study drug administration will be listed separately.

Participants in each analysis population will be tabulated and listed for all randomized participants.

Visit completion will also be summarized for all randomized participants.

7.2. Demographic and Other Baseline Characteristics

A summary of descriptive statistics for baseline and demographic characteristics will be reported for the safety population. Characteristics to be summarized include age (years), race, ethnicity, sex, body weight at screening (kg), height (m), and BMI (kg/m^2). These characteristics will be recorded at the time of the Screening visit. A corresponding listing will also be created.

Body weight reported in pounds will be converted to kg via body weight in pounds divided by 2.2. Height reported in inches will be converted to cm via height in inches multiplied by 2.54. BMI will be calculated automatically within the electronic data capture (EDC) system.

7.3. Prior and Concomitant Medications

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary (refer to the DSMP for the specific version). Medications reported on the case report form (CRF) will be categorized for analysis as prior, concomitant, or after study treatment by comparing the medication start and stop dates with the first and last dose of study medication dates. Prior medications will have both the medication start and stop dates prior to the first dose of study medication date. After medications will have both the medication start and stop dates after the last dose of study medication date. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study medication by at least one day.

The number and percentage of participants receiving prior and concomitant medications will be presented overall and by medication class. When reporting the number of participants receiving the medication, a participant will only be counted once if they ever received the medication within the medication class. Percentages will be based on the number of participants in the safety population.

Medication usage will be listed, including Anatomical Therapeutic Chemical (ATC) level 4 coding term, verbatim drug name, preferred drug name, start date, end date, dose (with unit), frequency, route, ongoing status and indication.

The following conventions will be used for imputing missing start dates.

- For start dates that are missing the day value with non-missing month and year:
  - The start day will be imputed as the first day of the non-missing start month.
- For start dates that are missing the day and month values with non-missing year:
  - If the start year is earlier than the year of Visit 1, the start month and day will be imputed as July 1.
  - If the start year is the same as the year of Visit 1, the start date will be set to the date of Visit 1.
  - If the start year is after the year of Visit 1, the start month and start day will be imputed as January 1.
- Completely missing start dates will be imputed as the date of Visit 1.

End dates will not be imputed.

7.4. Medical History

Medical history items will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA; refer to the DSMP for the specific version). Frequencies and percentages of participants with previous or current medical conditions or surgeries will be summarized by body system and dosing sequence for the safety population. A listing will also be created.

8. STUDY OPERATIONS

8.1. Protocol Deviations

Protocol deviations are defined in Section 15.3.1 of the protocol. All protocol deviations, including site level protocol deviations, will be listed by site with information such as type of deviation, severity of the deviation (major or non-major), date/day of occurrence, and the deviation details. Protocol deviations will be summarized in tabular format by type of deviation and dosing sequence.

As described in Section 5.5, protocol deviations will be used in the determining the composition of the per protocol population. Deviations resulting in exclusion from the per protocol population will be noted within the listing.

The severity of the deviation (major or non-major) will not be recorded in the CRFs by the sites. BARDA will review deviations to determine the severity of each, and this severity will be documented outside of the clinical database.

8.2. Blinding and Randomization

Eligible participants will be randomized at a 1:1 ratio to receive one of two treatment dosing sequences (A or B). Sequence A will receive sublingual (SL) atropine at Visit 1 followed by intramuscular (IM) atropine at Visit 2. Sequence B will receive IM atropine at Visit 1 followed by SL atropine at Visit 2. Randomization will be implemented per Section 10.7.1 of the protocol.

This is an open label study, and as such, the investigators, clinic personnel, and participants will not be blinded to the randomized treatment dosing sequence assignment. Rho, Allucent, and BARDA personnel will also not be blinded.

8.3. Measures of Treatment Compliance

Study drug administration adherence for each route of administration will be summarized and listed. Counts and percentages of participants receiving dose 1 and dose 2, separately, will be reported in tables; the denominator will be the number of participants within each group as randomized. For participants who receive both doses of study drug, the counts and percentages of subjects receiving both doses of study drug according to the correct dosing sequence will be summarized. For participants terminating study drug administration early, the reasons for terminating study drug early will also be presented.

Details will be listed for each administration, including date and time of administration, route received, whether full dosage was received, why full dose not received, and location of intramuscular administration/time held for sublingual administration.

9. EFFICACY EVALUATION

There are no protocol defined efficacy endpoints for this study. As such, this section is not applicable.

10. SAFETY EVALUATION

10.1. Overview of Safety Analysis Methods

All safety analyses will be carried out using the safety population defined in Section 5.3 unless otherwise noted.

Summary tables will present adverse events (AEs) summarized by route of administration and overall.

Listings will be prepared for all safety measurements. Unless noted otherwise in a specific section, all listings will be sorted in order of participant identifier (ID) and time of assessment (e.g., visit, time, and/or event).

10.2. Adverse Events

Any detrimental change in the participant's condition after signing informed consent and up to completion of the follow-up period after the last administration of study drug (i.e., through Follow-up [Day 15]) will be considered an AE and must be recorded.

All AEs will be classified by system organ class (SOC) and preferred term (PT), according to the Medical Dictionary for Regulatory Activities (MedDRA) version 27.0. The severity of AEs will be classified by the site investigator using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0. Relationship of AEs to study therapy (not related, unlikely related, possibly related, probably related, definitely related) will be assessed by the site investigator, based on their opinion. For summaries of study drug related AEs, the categories of "possibly related", "probably related", and "definitely related" will be considered study drug related. If the relationship is missing, it will be counted as "related" in summaries.

Treatment-emergent AEs are defined as AEs occurring after the participant received at least one dose of atropine. Treatment-emergent AEs will be identified as those with an onset date on or after the first dose of study medication.

If the start date of an AE is incomplete or missing, it will be assumed to have occurred on or after the first dose of investigational product, except if an incomplete date (e.g., month and year) clearly indicates that the event started prior to treatment.

The following conventions will be used for imputing missing start dates.

- For start dates that are missing the day value with non-missing month and year:
  - If the start year is the same as the year of first dose date, then do the following:
    - If the start month is the same as the month of first dose date, the start date will be imputed as the date of first dose date.

  - If the start month is not the same as the month of first dose date, then the start date will be imputed as the first day of the non-missing start month.
- If the start year is not the same as the year of first dose date, then the start day will be imputed as the first day of the start month.

- For start dates that are missing the day and month values with non-missing year:
  - If the start year is the same as the year of first dose date, the start date will be imputed as the date of first dose date.
  - If the start year is after the year of first dose date, the start month and start day will be imputed as January 1.
- Completely missing start dates will be imputed as the date of first dose date.

End dates will not be imputed.

If a participant experiences the same AE on multiple occasions, the event will be counted once for each occurrence when reporting the number of AEs. When reporting the number of participants experiencing the events, a participant will only be counted once if they experience at least one event within the particular SOC or PT. Percentages will be based on the number of participants in the safety population.

Additionally, when summarizing AEs by severity or relationship, the AE will be categorized according to the highest severity (or relationship) rating for that AE in that participant.

Each AE will be assigned to a route of administration based on the most recently received route of administration prior to the beginning of the adverse event. In order to standardize the follow-up time after each study route administration, summaries will include treatment-emergent AEs or serious AEs (SAEs) through 7 days after the last study route administration, inclusive of the study route administration day. In addition, treatment-emergent AEs and SAEs through study participation will be presented.

- An overall summary table of TEAEs throughout the study and through 7 days after last study drug dose will include:
  - Total number of TEAEs
  - Total number of TEAEs related to study drug
  - Total number of serious TEAEs
  - Total number of serious TEAEs related to study drug
  - Total number of severe (grade 3, 4, or 5) TEAEs
  - Total number of severe (grade 3, 4, or 5) TEAEs related to study drug
  - Total number of TEAEs leading to early termination of study drug

  - Total number of TEAEs leading to early study withdrawal
  - Total number of TEAEs leading to death

- TEAEs classified by MedDRA SOC and PT will be summarized for each route of administration and overall for all TEAEs throughout the study and through 7 days after last study drug dose, separately.
- TEAEs classified by MedDRA SOC, PT, and maximum severity will be summarized for each route of administration and overall for all TEAEs throughout the study and through 7 days after last study drug dose, separately.
- TEAEs classified by MedDRA SOC, PT, and strongest relationship will be summarized for each route of administration and overall for all TEAEs through 7 days after last study drug dose.
- TEAEs classified by MedDRA SOC and PT will be summarized for each route of administration and overall for all serious TEAEs throughout the study and through 7 days after last study drug dose, separately.
- TEAEs classified by MedDRA SOC and PT will be summarized for all TEAEs leading to early termination of study drug, study withdrawal, and death, separately.

Corresponding listings will be created for the above-mentioned tables.

10.3. Deaths, Serious Adverse Events, and Other Significant Adverse Events

Deaths and other serious or significant adverse events will be encompassed by the various adverse event types defined in Section 10.2, and these events will be summarized and listed as described in that section.

A separate listing will be created for all deaths summarizing the time to death and cause of death.

10.4. Clinical Laboratory Evaluation

Virus serology for human immunodeficiency virus, HBsAg, and anti-HCV antibody collection date and results at screening will be listed for all participants.

Clinical chemistry (including liver function), hematology, and thyroid-stimulating hormone laboratory results at screening will be listed, as well as all results that may be collected for early termination or unscheduled visits.

10.5. Vital Signs, Physical Findings, and Other Observations Related to Safety

10.5.1. Vital Signs

Vital signs parameters of blood pressure (systolic and diastolic; mmHg), heart rate (beats/minute), respiratory rate (breaths/minute), oral temperature (°F), and weight (kg) will be

summarized for Screening, and pre-dose for Visit 1 and Visit 2 by dosing sequence. For all results collected post-baseline, change from baseline will be displayed. Visit 1 pre-dose vital signs results will be considered baseline for summaries of vital signs data. If the Visit 1 pre-dose result is missing, screening results will be considered baseline.

Separately, blood pressure and heart rate will be collected every 15 minutes for the first hour post-dose, every 20 minutes for the second hour post-dose, and every 30 minutes for the third and fourth hour post-dose. These results will be summarized by dose received. Change from baseline will be summarized, with baseline being defined as the pre-dose value for the applicable dose received.

All vital signs will be listed in by- participant listings including visit and collection date.

10.5.2. Physical Examinations

A physical examination will be performed at the Screening Visit to assess and confirm eligibility. The examination will include a general assessment of the skin, head, ears, eyes, nose, throat, neck, oral cavity, thyroid, lungs, heart, abdomen, lymph nodes, and musculoskeletal system/extremities, and a neurological examination. A symptom-based physical examination may be performed at dosing visits, or at an Unscheduled or ET Visit per the investigator's discretion.

In general, clinically significant abnormal findings are expected to be associated with an item recorded in the participant's medical history (when detected at Screening) or as an AE (when detected after Screening). Physical exam results will not be reported otherwise.

10.5.3. Other Safety Measures

10.5.3.1 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be recorded and assessed at the Screening Visit, Visit 1 (pre-dose), and Visit 2 (pre-dose). Additionally, an ECG may be performed for any complaints of chest discomfort, lightheadedness, and/or palpitations per the investigator's discretion and repeated as needed during Visit 1 (post-dose) and Visit 2 (post-dose). ECGs may also be performed at an Unscheduled or ET Visit per the investigator's discretion. Results will be reported in data listings, including visit and collection date.

10.5.3.2 Pregnancy Assessments

Pregnancy assessments will be done at Screening and pre-dose Visit 1 and Visit 2. Date, time, and results of each pregnancy assessment will be listed for all female participants. In the case of one or more pregnancies occurring during study participation, a data listing will be prepared to display applicable information, including date reported, method of pregnancy confirmation, delivery date (if applicable), pregnancy termination status and week (if applicable), and any problems or congenital abnormalities present for each birth.

11. PHARMACOKINETIC EVALUATION

All pharmacokinetic (PK) analyses will be performed on the PK analysis population and the per protocol population (if the per protocol population differs from the PK analysis population).

The primary analyses will be performed on the PK analysis population. All PK analyses performed on the per protocol population will be considered supportive analyses.

Subject time-concentration data without evaluable results will be excluded from analyses on the PK analysis population separately for each PK parameter.

Actual PK sample collection times will be used in analyses. If a collection time is incomplete or missing, the nominal time will be used for analyses.

Atropine concentrations marked as "not detectable" will be imputed as half of the lower limit of quantitation in analyses.

11.1. Pharmacokinetic Endpoints

The following PK parameters contribute to the analysis of the primary endpoint for this study:

- Area under the analyte concentration versus time curve to infinity (AUC_{∞})
- Area under the analyte concentration versus time curve to time of last quantifiable data point (AUC_t)

Additionally, the following PK parameters contribute to the analysis of the secondary endpoints for this study:

- Area under the analyte concentration versus time curve to 45, 60, 90, 120, 150, and 240 minutes (AUC_{45}, AUC_{60}, AUC_{90}, AUC_{120}, AUC_{150}, and AUC_{240}, respectively)
- Maximum measured plasma concentration (C_{max})
- Time to C_{max} (t_{max})
- Apparent terminal elimination half-life ($t_{½}$)
- Terminal elimination rate constant (λ_z)
- Volume of distribution (V_d/F)
- Total body clearance (CL/F)
- Absorption rate constant (K_a)

Additional PK parameters may be resulted for exploratory purposes.

11.2. Pharmacokinetic Methods

11.2.1. Pharmacokinetic Endpoint Estimation

Phoenix WinNonLin software Version 8.3 or later will be used to generate PK parameter estimates[3].

The PK parameters will be estimated for each subject and route of administration using the noncompartmental method for extravascular dosing routes, using the linear log trapezoidal rule and uniform weighting for plasma models.

The terminal elimination rate constant (λz) will be calculated using the best fit method. Parameters AUC_{∞}, $t_{½}$, CL/F and V_d/F will be non-evaluable when there are fewer than 3 data points beyond t_{max} in the time-concentration curve.

The absorption rate constant (K_a) for each route of administration will be computed as a slope over a user-defined range after review of subject specific time-concentration profiles. Rate constants may also be calculated using the best-fit method for linear regression in addition to user-defined ranges.

11.2.2. Statistical Analysis of Pharmacokinetic Endpoints

PK parameters will be summarized by route of administration using descriptive statistics on the scales outlined below.

For the PK parameters AUC_{∞}, AUC_t, AUC_{45}, AUC_{60}, AUC_{90}, AUC_{120}, AUC_{150}, and AUC_{240}, and C_{max}, the following will be displayed in the summary table:

a. Count of subjects with non-missing data for the applicable analysis

b. Geometric mean

c. CV% of the geometric mean, which is calculated using the following equation:

$$\sqrt{10^{\ln(10)x(SD(log_{10}value))^2} - 1} \text{ x } 100$$

d. Minimum and maximum values

e. Median, and 1st and 3rd quartile values

For the PK parameters t_{max}, $t_{½}$, λ_z, CL/F, V_d/F, and K_a the following descriptive summary statistics will be reported on the untransformed scale:

a. Count of subjects with non-missing data for the applicable analysis

b. Arithmetic mean

c. Standard deviation

d. Minimum and maximum values

e. Median, and 1st and 3rd quartile values

Bioavailability of SL administration of atropine sulfate relative to that of IM atropine sulfate will be assessed based on ratios of least-square means and their associated 90% confidence intervals (CIs) generated from linear mixed models to the log-transformed PK parameters including terms for dosing sequence, route (SL or IM), and period as fixed effects, and subject nested within dosing sequence as a random effect. No adjustment will be made for dosing cohort or the interaction for dosing cohort and route.

Ratios of least-squares means of key plasma PK parameters and associated 90% CIs from the linear mixed model will be anti-log transformed to obtain estimates on the original scale.

Bioequivalence will be considered met if the 90% CI of the ratio for AUC_{∞} and AUC_t lie within 80.00 to 125.00%.

Subject specific and population time-concentration profiles on both log and linear scales will be presented. Additionally, PK parameters will be listed by route of administration and subject.

As a supportive analysis of bioavailability, descriptive summary statistics will be displayed in tables for subject-specific ratios between routes of administration.

For each PK parameter, boxplots will be displayed for each route of administration with subject specific lines overlaid.

12. INTERIM ANALYSES AND DATA MONITORING

No interim analysis is planned for this study.

In the event a study pausing rule is met, the medical monitor and the BARDA Pharmacovigilance SME and/or BARDA Medical Officer will convene to discuss and review relevant cases. No pre-planned interim safety reviews will be conducted for this study.

13. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

The safety analysis population was updated to remove “are randomized and” to account for anyone who received any study drug but was not randomized. This will ensure these individuals still get summarized in safety displays.

14. REFERENCES

1. International Council for Harmonisation (ICH) guidelines, efficacy. May 2021.

2. SAS Institute Inc. 2023. SAS/STAT® 15.3 User's Guide. Cary, NC: SAS Institute Inc.

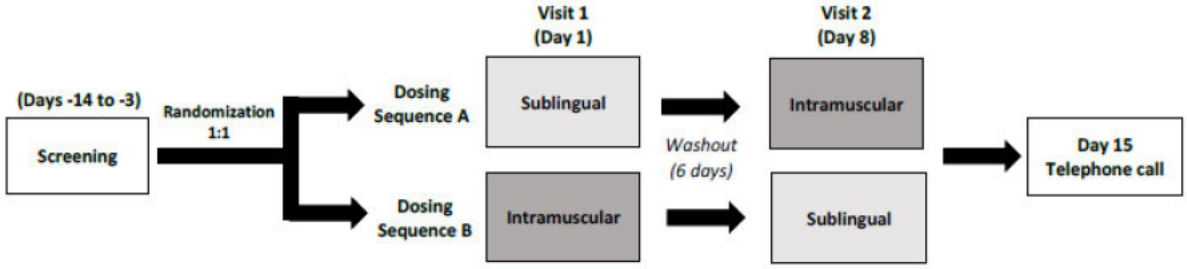
3. Phoenix WinNonLin® User's Guide, version 6.4, Pharsight Corporation.

15. APPENDIX

15.1. Study Flow Chart

Figure 1 presents a diagram of the overall study design.

Figure 1: Overall Study Design



15.2. Schedule of Events

The schedule of events is contained in Appendix 2 of the protocol.

16. ATTACHMENTS

Table, figure, and listing shells will be prepared as a separate document to this SAP.