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1 Title

Full Study Title
Absolute Bioavailability and Pharmacokinetics of Sublingual Atropine Sulfate Ophthalmic Solution USP 1% (0.5 mg and 1.0 mg) Compared with Intravenous Atropine Sulfate (1.0 mg) in Healthy Adults: A Randomized, Open-Label, Single-Dose, Three-Sequence, Three-Period Crossover Study [United States]

Study Acronym
SLATE-BA (Sublingual Atropine Bioavailability Evaluation)

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- None specified

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- IND: Conducted under U.S. IND regulations (21 CFR 312); IND number not stated in this protocol [1]
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- Pharmacovigilance/24-hour Safety Contact: To be designated; Contact: To be provided

Regulatory and Ethical Compliance
This study will be conducted in accordance with 21 CFR 312 and applicable U.S. regulations and guidance, the principles of ICH E6(R2) Good Clinical Practice, and the ethical principles originating in the Declaration of Helsinki [1,2].

References

1. Electronic Code of Federal Regulations (eCFR). Title 21—Food and Drugs; Part 312—Investigational New Drug Application. Silver Spring (MD): U.S. Food and Drug Administration; [cited 2026 Oct 2]. Available from: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-D/part-312
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2 INTRODUCTION

2.1 Study Rationale

Overview and justification

Timely antimuscarinic therapy is a cornerstone of medical management for organophosphorus nerve agent and carbamate pesticide poisoning, with atropine recommended as first-line treatment to mitigate life-threatening muscarinic manifestations including bronchorrhea, bronchospasm, and bradycardia [1]. In mass-casualty settings, supply, training, or access constraints can delay parenteral administration. Repurposing a widely distributed, room-temperature–stable atropine sulfate ophthalmic solution (USP 1%) for sublingual administration could provide a rapidly deployable, needle-free countermeasure to bridge care until definitive treatment is available. However, regulatory and clinical deployment require evidence that sublingual administration achieves clinically relevant systemic exposure and an exposure–time profile consistent with therapeutic intent. An absolute bioavailability study directly comparing sublingual dosing with an intravenous (IV) reference is the accepted approach to characterize and quantify the fraction of the dose reaching the systemic circulation and to contextualize rate and extent of exposure [2,3].

Atropine exhibits rapid distribution with a terminal elimination half-life generally in the 2–4 hour range in healthy adults after IV administration, supporting a crossover design with adequate washout and intensive sampling to characterize early absorption and distribution phases [4,5]. While clinical experience and small studies suggest that atropine ophthalmic drops administered sublingually can reduce secretions and increase heart rate, indicating clinically meaningful systemic absorption via the oral mucosa, quantitative pharmacokinetic (PK) characterization and absolute bioavailability estimates for this route and formulation remain limited or absent in healthy adults [6–8]. Establishing the absolute bioavailability and PK of sublingual atropine at clinically relevant single doses (0.5 mg and 1.0 mg) compared with 1.0 mg IV will fill this evidence gap, inform dose selection for emergency use, and support risk–benefit assessments and labeling or guidance development as appropriate.

Alignment with regulatory expectations and analytical needs

FDA guidance underscores that absolute bioavailability should be determined by comparing systemic exposure after extravascular administration with exposure after IV administration of the same active moiety, using validated bioanalytical methods and PK endpoints including AUC, Cmax, tmax, and half-life [2]. A randomized, single-dose, three-period crossover in healthy adults with a 1.0 mg IV reference is consistent with these recommendations and enables within-subject comparisons to reduce variability and support precise estimation of exposure ratios and absorption kinetics [2,3]. The selected sublingual doses are within the approved adult therapeutic dosing range for atropine used parenterally for bradycardia and poisoning management [9,10], while balancing safety in healthy volunteers. The study's descriptive comparative framework (not a formal bioequivalence test) is appropriate because the aim is to quantify absolute bioavailability and characterize route-related exposure, not to establish generic substitutability [2,3].

How the study complements existing knowledge

Nonclinical and clinical literature demonstrates that the sublingual route can provide rapid systemic absorption for small, lipophilic or tertiary amines via the oral mucosa, bypassing first-pass metabolism [3]. Atropine, a tertiary amine, has shown systemic effects after sublingual or oral mucosal

administration in clinical contexts such as sialorrhea management, supporting biological plausibility for effective transmucosal delivery from an ophthalmic solution [6–8]. Nevertheless, existing reports are not designed to deliver definitive absolute bioavailability, dose-normalized exposure comparisons versus IV, or early absorption profiling required to inform countermeasure deployment. By employing dense early sampling (2–60 minutes) and an IV reference, this study will define the rate and extent of sublingual absorption, estimate absolute bioavailability for 0.5 mg and 1.0 mg doses, and explore dose proportionality across the sublingual dose range, thereby directly informing operational and dosing guidance for field use. This knowledge will also support translational modeling of exposure–response for heart rate and xerostomia as early pharmacodynamic markers, informing both safety monitoring and potential clinical performance benchmarks in future studies. These interpretive linkages reflect synthesis across the cited sources and are explicitly hypothesis-driven.

Study hypothesis

- Primary hypothesis: Sublingual administration of atropine sulfate ophthalmic solution USP 1% at 0.5 mg and 1.0 mg yields measurable systemic exposure with quantifiable absolute bioavailability relative to 1.0 mg IV atropine, and produces a time to peak concentration consistent with a rapid-onset countermeasure intent in healthy adults [2,6–8].
- Safety hypothesis: Single sublingual doses of 0.5 mg and 1.0 mg are safe and generally well tolerated in healthy adults, with antimuscarinic adverse events (for example, xerostomia, tachycardia) that are transient and manageable under clinical monitoring, consistent with atropine's established safety profile at comparable therapeutic doses [9,10].

Secondary and exploratory objectives (study-linked rationale)

- Secondary objectives (evidence-linked rationale):

- To describe the safety and tolerability of 0.5 mg and 1.0 mg sublingual atropine, including changes in xerostomia and vital signs, aligning with label-identified antimuscarinic effects and cardiovascular warnings for atropine [9,10].

- Exploratory objectives (interpretive, hypothesis-driven):

- To explore dose proportionality between 0.5 mg and 1.0 mg sublingual atropine using exposure metrics (AUC, Cmax) to inform dose selection for future operational use [2].
- To explore exposure–response relationships between plasma atropine concentrations and short-term pharmacodynamic markers (heart rate elevation and xerostomia scores) to inform clinical monitoring parameters in emergency deployment scenarios [1,9,10].
- To characterize inter- and intra-subject variability in sublingual absorption to anticipate performance in real-world conditions (for example, variable salivary flow), supporting countermeasure planning [3,6–8]. These exploratory aims are interpretive and will be analyzed descriptively.

Appropriateness of the selected design

A randomized, open-label, single-dose, three-sequence, three-period crossover with approximately 7-day washouts is suitable for an atropine half-life of about 2–4 hours, providing >5 half-lives between periods to preclude carryover [2,4,5]. The IV period provides the reference for calculating absolute bioavailability (AUCextravascular/AUCIV × doseIV/doseextravascular). Intensive early sampling enables accurate estimation of Cmax and tmax after sublingual dosing and robust AUC extrapolation to infinity for all routes [2]. Conducting the study in healthy adults reduces pathophysiologic and concomitant-therapy variability, is standard for BA/BE investigations, and is consistent with EMA and FDA expectations for exploratory or pilot BA studies [2,11].

Eligibility criteria and their rationale

Eligibility focuses on enrolling healthy adults while minimizing foreseeable atropine-related risk and confounding of PK assessments, consistent with labeling contraindications/precautions and BA guidance [2,9,10].

Inclusion criteria	Rationale
Healthy males and non-pregnant, non-breastfeeding females aged 18–55 years	Standard BA population to minimize variability and risk in a nontherapeutic study [2,11]
Medically healthy by history, exam, vitals, 12-lead ECG, and clinical labs	Ensures safety and reliable PK characterization [2]
Women of childbearing potential using highly effective contraception	Mitigates embryo-fetal risk consistent with ethical conduct in healthy volunteers [2]
Ability to comply with restrictions (for example, nothing by mouth 1 hour pre/postdose)	Reduces variability in sublingual absorption [2,3]

Exclusion criteria	Rationale
Clinically significant cardiovascular disease, arrhythmia, or conduction abnormality; screening QTc ≥450 ms (males) or ≥470 ms (females); uncontrolled hypertension (>140/90 mmHg)	Atropine can precipitate tachycardia and arrhythmias; exclude those at increased risk [9,10]
Angle-closure glaucoma or history of intraocular surgery predisposing to angle closure	Antimuscarinics can increase intraocular pressure; contraindicated in angle-closure glaucoma [9,10]
Gastrointestinal motility disorders (for example, obstruction, severe constipation, ileus), or severe GERD	Antimuscarinic effects can worsen GI hypomotility; avoid confounding and risk [9,10]
Urinary retention or clinically significant prostatic hypertrophy	Risk of urinary retention increased with antimuscarinics [9,10]
Pre-existing xerostomia or salivary gland disorders	Can confound xerostomia assessments and tolerability endpoints [9,10]
Myasthenia gravis (unless receiving anticholinesterases for therapeutic reasons, which are not applicable in healthy volunteers)	Antimuscarinics may exacerbate weakness; risk outweighs benefit in healthy subjects [9,10]
Significant hepatic or renal impairment	May alter drug handling and safety; exclude to minimize risk and PK variability [2]
Known hypersensitivity to atropine or belladonna alkaloids	Contraindication [9,10]
Positive tests for HIV, HBV, or HCV; active infection	Safety and confounding concerns for Phase 1 studies [2]
Prescription medications within 30 days or recent drugs altering heart rate/salivation; substance or alcohol abuse	Avoids drug interactions and PD confounding [2,9,10]
Tobacco or nicotine use that could alter salivation	Minimizes variability in sublingual absorption [3]

Planned sample size and rationale

A target of approximately 15 enrolled participants to obtain about 12 evaluable completers across three sequences is consistent with regulatory expectations for pilot bioavailability characterization and is adequate for descriptive estimation of central tendency and variability for AUC and Cmax in a within-subject crossover without formal hypothesis testing [2,11]. This size balances precision needs for exposure ratio estimation with ethical exposure to antimuscarinic effects in healthy volunteers, while accommodating an anticipated dropout rate of approximately 20 percent.

Anticipated contribution to development

Quantifying the absolute bioavailability and early absorption kinetics of sublingual atropine from an FDA-approved ophthalmic solution, alongside safety and tolerability profiling in healthy adults, will provide foundational data to inform dosing, operational use, and potential labeling or guidance development for a needle-free countermeasure in nerve agent incidents. The study's results will also support exposure-response modeling and the design of subsequent clinical or operational studies assessing field performance and clinical outcomes. These forward-looking applications are

interpretive and contingent on the descriptive PK and safety outcomes generated here, integrated with established clinical guidance for atropine use in poisoning and bradycardia [1,9,10].

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2.2 Background Information

2.2.1 Nonclinical Pharmacology and Pharmacokinetics

Summary of nonclinical pharmacology

Atropine is a tertiary amine antimuscarinic that competitively antagonizes acetylcholine at muscarinic receptors, producing dose-dependent reductions in secretions, vagolytic increases in heart rate, and smooth muscle relaxation in multiple organ systems. These pharmacodynamic effects are rapid after parenteral dosing and correlate temporally with systemic exposure in animals and humans, supporting the use of concentration–time metrics to interpret early pharmacodynamic markers such as heart rate and xerostomia in this protocol [1,2]. Nonclinical data demonstrate central and peripheral distribution, with the ability to cross the blood–brain and placental barriers, features attributable to its tertiary amine structure and moderate lipophilicity [1,3].

Physicochemical and formulation considerations relevant to sublingual absorption

Atropine (free base) has reported basic pKa values near 9.7 and a logP of approximately 1.8–2, conferring pH-dependent ionization and moderate membrane affinity; as a weak base, the unionized fraction increases with higher local pH, which can favor transmucosal permeation [4]. The test product is an aqueous 1% atropine sulfate ophthalmic solution (10 mg/mL) formulated with excipients typical of ophthalmic preparations (for example, benzalkonium chloride as preservative) and an acidic pH range specified on labeling to maintain solubility and stability; these attributes may increase ionization in saliva and could modestly slow transcellular flux, whereas the high drug concentration, direct mucosal placement, and avoidance of gastrointestinal transit and hepatic first-pass metabolism favor measurable sublingual absorption [3,5]. General buccal/sublingual delivery principles indicate that residence time at the mucosa, salivary flow, and swallowing frequency materially influence the rate and extent of absorption; the study's nil-per-os window and standardized administration conditions are aligned with these determinants [3]. This paragraph reflects interpretive synthesis of established mucosal delivery principles with the labeled characteristics of the ophthalmic formulation.

Absorption

After intravenous administration in humans, atropine exhibits rapid distribution with early plasma concentration decline and a terminal elimination half-life generally in the 2–4 hour range, enabling precise characterization of disposition phases and supporting a crossover design with ≥7-day washouts for this protocol [1,2]. In extravascular contexts, avoidance of first-pass metabolism via the sublingual route is expected to increase systemic exposure relative to swallowed drug, and oral mucosal permeability for small, moderately lipophilic, tertiary amines is well established in nonclinical systems and translational reviews [3]. Although quantitative absolute bioavailability data for sublingual atropine are limited, nonclinical and clinical experience with atropine's rapid onset after mucosal exposure, coupled with its physicochemical profile, supports the expectation of prompt, measurable systemic concentrations following sublingual dosing in healthy adults; the dense early sampling in this study (2–60 minutes) specifically addresses absorption kinetics and peak timing [1–3].

Distribution

Atropine demonstrates extensive tissue distribution in animals and humans, with reported apparent volumes of distribution consistent with rapid partitioning into highly perfused organs and subsequent redistribution, and with demonstrable penetration into the central nervous system [1,2]. Plasma protein binding is low to moderate, facilitating prompt equilibration between plasma and effect sites and contributing to the observed early pharmacodynamic effects after systemic exposure [1].

Metabolism and excretion

Atropine undergoes hepatic metabolism, including hydrolysis and conjugation pathways, with renal excretion of unchanged drug and metabolites; a substantial fraction of dose is recovered in urine within 24 hours in humans, consistent with overall clearance dominated by hepatic biotransformation and renal elimination of parent and metabolites [1,2]. These pathways do not suggest clinically meaningful transporter limitations for single-dose studies, and no saturable elimination has been described in the dose range relevant to this protocol [1,2]. Accordingly, single sublingual doses of 0.5 mg and 1.0 mg are expected to yield linear, dose-proportional exposure within the studied range, an expectation evaluated exploratorily in the present design based on total exposure (AUC) and peak concentration (Cmax). This statement is interpretive, grounded in the cited human disposition literature.

Nonclinical factors potentially influencing bioavailability

- Mucosal pH and ionization: As a weak base, atropine's unionized fraction increases at higher pH, which can enhance transcellular flux; conversely, acidic microenvironments favor ionization and may reduce permeability [3,4,5].

- Contact time and salivary dynamics: Saliva production and swallowing can shorten residence time and dilute drug at the absorption surface; standardized nil-per-os conditions minimize this effect during the early absorption window [3].
- Formulation attributes: The ophthalmic solution's aqueous, preservative-containing composition and high concentration support rapid wetting and drug availability at the sublingual surface; ophthalmic isotonicity and low viscosity may favor spread but may also increase the likelihood of inadvertent swallowing if administration is not controlled, reinforcing the protocol's dosing technique and postdose restrictions [5]. These considerations are interpretive syntheses informed by general transmucosal delivery principles and product labeling.

Implications for clinical bioavailability design and dose selection

Collectively, nonclinical pharmacology and human disposition data support: 1) selection of a 1.0 mg IV reference to define absolute bioavailability; 2) intensive early sampling to resolve sublingual absorption and peak timing; 3) single sublingual doses of 0.5 mg and 1.0 mg within the therapeutic range to characterize dose proportionality while limiting antimuscarinic adverse effects; and 4) ≥7-day washouts to preclude carryover given the 2–4 hour terminal half-life and extensive distribution [1,2,5]. Explicit control of pre-/postdose oral intake and standardized sublingual administration are aligned with established determinants of mucosal absorption and are expected to reduce variability in the rate and extent of exposure [3,5].

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2.2.2 Nonclinical Safety

Overview

The active moiety atropine sulfate has extensive clinical use as an antimuscarinic agent. For an alternate administration route using a marketed ophthalmic solution (USP 1%) applied sublingually, regulatory guidance indicates that when the active ingredient and total systemic exposure are within the range of an approved product with well-characterized human safety, additional nonclinical toxicity studies are generally not required beyond a scientific assessment of local tolerance and excipient risks for the new route [1,2]. Accordingly, the nonclinical safety summary below focuses on established toxicology for atropine and on formulation/excipient considerations relevant to brief sublingual exposure at the proposed single doses.

Acute and repeat-dose toxicity

Across animal toxicology literature and poison monographs, atropine's acute toxicity is characterized by dose-dependent antimuscarinic pharmacology (tachycardia, mydriasis, xerostomia, hyperthermia,

CNS excitation) without a consistent pattern of structural target-organ injury at exposures many fold higher than therapeutic human doses; mortality in animals is associated with severe pharmacologic exaggeration rather than specific organ necrosis [3]. Repeat-dose toxicology data similarly indicate that findings are attributable to sustained antimuscarinic effects (e.g., reduced secretions, gastrointestinal hypomotility, tachycardia), with no definitive target-organ pathology identified at sublethal exposures in standard species; recovery occurs after discontinuation, consistent with reversible receptor antagonism [3]. This profile aligns with human labeling, in which safety concerns reflect predictable antimuscarinic pharmacodynamics rather than organ-specific toxicity [4].

A definitive animal no-observed-adverse-effect level (NOAEL) applicable to brief sublingual exposure is not identified in publicly available regulatory labeling. However, the proposed single sublingual doses (0.5 mg and 1.0 mg) and the 1.0 mg IV reference fall within approved adult therapeutic dosing used clinically, for which human safety is established under monitoring [4,5]. This supports a wide nonclinical safety margin for transient, single-dose exposure in healthy adults as implemented in this bioavailability study. This conclusion reflects interpretive synthesis of the cited sources.

Genotoxicity, carcinogenicity, and reproductive toxicity

Regulatory labeling indicates that long-term carcinogenicity studies and standard genotoxicity batteries have not been comprehensively conducted for atropine sulfate; available nonclinical information does not identify a genotoxic signal, but the dataset is limited [4,5]. Adequate animal reproduction studies with atropine sulfate are not described in labeling; human data in pregnancy are insufficient to determine a drug-associated risk, and use is guided by clinical necessity [5]. In this healthy volunteer study, women of childbearing potential are required to use highly effective contraception, and only single doses are administered, in line with ICH M3(R2) expectations for minimizing reproductive risk in early clinical studies without disease benefit [2,5].

Local tolerance and excipient considerations for sublingual use

The test article is a commercially available 1% atropine sulfate ophthalmic solution that typically contains benzalkonium chloride (BAC) as a preservative at concentrations around 0.01% as indicated on product labeling [6]. With administration volumes of 50–100 µL, the absolute BAC exposure is in the microgram range and is expected to pose minimal systemic risk; transient local irritation or taste disturbance is possible based on general cationic surfactant properties and ophthalmic experience, but clinically significant oral mucosal injury is not anticipated with a single brief exposure [1,6]. No formulation-specific toxicities beyond antimuscarinic pharmacology are identified in labeling for the ophthalmic or injectable atropine products [4–6]. Consistent with FDA guidance on alternate routes, the brief contact time, small dose volume, and use of an approved aqueous solution mitigate local tolerance concerns; clinical monitoring in this protocol will detect any unexpected mucosal effects [1]. This paragraph reflects interpretive synthesis of route-of-administration guidance with product labeling.

Safety margin relative to projected human exposure

- Proposed exposures: 0.5 mg and 1.0 mg sublingual atropine sulfate (ophthalmic solution) and 1.0 mg IV atropine sulfate.
- Clinical comparators: These doses are within the labeled therapeutic range for atropine used parenterally in adults for bradycardia and antimuscarinic indications [5]. Thus, the study's projected exposures lie within established human safety experience, providing a clinically derived safety margin that obviates reliance on an animal NOAEL for single-dose administration in healthy volunteers [1,2,5]. This conclusion is interpretive, integrating regulatory guidance with labeling.

Target-organ toxicities and identified risks

No target-organ toxicities have been identified for atropine in standard nonclinical sources beyond reversible, exaggerated antimuscarinic pharmacology. Anticipated risks are pharmacodynamic and

include tachycardia, decreased secretions/xerostomia, mydriasis/blurred vision, urinary hesitancy, and, at higher exposures, CNS excitation; these effects are well described in human labeling and will be monitored [4,5]. No excipient-related systemic toxicities are expected at the administered micro-volumes; potential local tolerability findings are limited to mild oral mucosal irritation or unpleasant taste with BAC-containing ophthalmic solutions [1,6].

Conclusion

Based on authoritative nonclinical information and extensive human experience with atropine at comparable single therapeutic doses, together with FDA and ICH guidance for alternate-route use of an approved formulation, the available data support the safety of administering atropine sulfate ophthalmic solution 1% sublingually at 0.5 mg and 1.0 mg under clinical monitoring in healthy adults, and of the 1.0 mg IV reference dose, for the purposes of this randomized, single-dose bioavailability study [1,2,4–6].

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2.2.3 Summary of Clinical Pharmacokinetics of [investigational product]

Overview of existing human pharmacokinetics

Atropine displays rapid distribution following intravenous (IV) administration with a multi-exponential decline in plasma concentrations and a terminal elimination half-life generally in the 2–4 hour range in healthy adults [1–3]. Disposition is characterized by extensive tissue distribution and low to moderate plasma protein binding, with clearance mediated by hepatic metabolism and renal excretion of parent and metabolites; a substantial fraction of dose is recovered in urine within the first 24 hours [1–3]. These properties support precise characterization of exposure using noncompartmental methods in crossover designs and enable robust estimation of absolute bioavailability when an IV reference is used.

Intravenous reference pharmacokinetics (dose context for this protocol)

Across classic human studies and labeling, typical parameter ranges after single IV doses in healthy adults include:

- Time to maximum concentration (tmax): occurs at end of IV administration (no absorption phase) [1–3].

- Maximum concentration (Cmax): proportional to dose and distribution volume; early distributive phase leads to rapid decline from the post-infusion peak [2,3].
- Systemic exposure (AUC): increases approximately proportionally with dose in the therapeutic range [2,3].
- Terminal elimination half-life (t½): approximately 2–4 hours [1–3].
- Apparent volume of distribution: on the order of 1–3 L/kg, consistent with extensive tissue distribution [2,3].
- Clearance: on the order of 1–3 mL/min/kg, reflecting combined metabolic and renal pathways [2,3].

These IV benchmarks provide the denominator for calculating absolute bioavailability and for interpreting sublingual rate and extent of absorption in the present study.

Extravascular administration: oral and sublingual context

Oral administration of atropine is associated with first-pass loss and variable gastrointestinal absorption, leading to slower onset and lower systemic exposure relative to parenteral dosing; by contrast, transmucosal delivery via the sublingual route can bypass first-pass metabolism and provide more rapid entry to the systemic circulation for small, moderately lipophilic tertiary amines such as atropine [1,4,5]. Clinical reports using atropine ophthalmic drops placed sublingually demonstrate systemic antimuscarinic effects (reduced drooling, increased heart rate), indicating that clinically relevant absorption can occur via the oral mucosa, although quantitative plasma pharmacokinetics and absolute bioavailability estimates for this route and formulation in healthy adults are limited or absent [4,6–8]. These observations support biological plausibility for measurable sublingual exposure while underscoring the need for definitive, IV-referenced pharmacokinetic characterization.

Variability and known modifiers

In healthy adults, interindividual variability in atropine disposition after IV dosing is typically moderate, reflecting differences in distribution volume and clearance; within-subject variability is lower, supporting crossover evaluation of exposure ratios [2,3]. Intrinsic factor effects on atropine pharmacokinetics have not been comprehensively quantified in controlled studies; labeling emphasizes clinical sensitivity differences (e.g., in older adults) more than definitive pharmacokinetic shifts, and no transporter-mediated interactions of clinical relevance have been described for single-dose use [1]. For extravascular administration, extrinsic factors influencing sublingual absorption include saliva production and swallowing frequency, residence time of the formulation under the tongue, and local pH, all of which can alter the rate and extent of exposure; standardizing nil-per-os conditions around dosing is consistent with established principles for buccal/sublingual delivery and is intended to reduce variability in this protocol [5]. This paragraph reflects interpretive synthesis anchored in the cited sources.

Summary of typical clinical pharmacokinetic parameters and expectations for the present study

Based on human IV data and translational principles for transmucosal delivery, the following expectations guide the current bioavailability assessment:

- Rapid absorption after sublingual dosing with measurable plasma concentrations within minutes, a tmax anticipated within approximately 10–60 minutes, and a terminal t½ similar to IV (disposition-driven), enabling reliable estimation of AUC0–∞ and Cmax using dense early sampling [1–3,5]. This expectation is interpretive, integrating IV disposition with mucosal delivery principles.
- Approximately dose-proportional increases in Cmax and AUC between 0.5 mg and 1.0 mg sublingual doses within the studied range, absent evidence of saturable elimination at therapeutic exposures [2,3].
- Moderate intersubject variability in Cmax and AUC for the sublingual route, potentially greater than IV due to absorption-related factors (residence time, salivary dynamics), justifying within-subject comparisons in a crossover design [2,5].

Remaining data gaps and how the present study addresses them

- Absolute bioavailability of atropine delivered sublingually from a commercially available 1% ophthalmic solution in healthy adults has not been quantified against an IV reference; existing reports document clinical effects but lack plasma PK and AUC-based bioavailability estimates [4,6–8]. The present study provides definitive AUC-based absolute bioavailability for 0.5 mg and 1.0 mg sublingual doses relative to 1.0 mg IV, with calculation of exposure ratios and confidence intervals.
- Early absorption kinetics and peak timing after sublingual dosing are insufficiently characterized to inform rapid-onset countermeasure use. Intensive sampling from 2 to 60 minutes postdose will resolve the absorption phase, enabling precise estimation of Cmax and tmax.
- Dose proportionality across clinically relevant sublingual doses has not been described. Descriptive comparisons of exposure metrics (AUC, Cmax) between 0.5 mg and 1.0 mg sublingual doses will explore proportionality within the studied range.
- Variability drivers specific to sublingual administration (e.g., salivary flow) are not quantified in prior clinical PK work. Standardized administration and pre/postdose restrictions in a crossover framework will enable estimation of inter- and intra-subject variability for sublingual absorption.

Collectively, prior human disposition data for atropine and translational evidence for mucosal delivery support the feasibility of achieving clinically relevant systemic exposure via the sublingual route, while the lack of quantitative, IV-referenced pharmacokinetic data for the ophthalmic 1% solution administered sublingually constitutes a central evidence gap that this absolute bioavailability study is designed to fill [1–8].

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2.3 Benefit–Risk Assessment

Anticipated benefits

This randomized, single-dose, three-period crossover study will quantify the absolute bioavailability of

atropine delivered sublingually from an approved 1% ophthalmic solution by direct comparison with a 1.0 mg intravenous (IV) reference using validated pharmacokinetic (PK) endpoints (AUC, Cmax, tmax, t½). FDA and EMA guidance identify IV-referenced absolute bioavailability studies as the appropriate approach to determine the fraction of dose reaching the systemic circulation and to contextualize absorption rate and extent for alternative routes, thereby generating data essential for regulatory decision-making and development planning [1,2]. In the mass-casualty context of organophosphorus/carbamate nerve agent exposures, rapidly deployable, needle-free atropine could bridge care when injections are delayed; definitive PK comparability and absolute bioavailability estimates are foundational to support dosing recommendations and potential policy or labeling considerations for such an alternate route [3]. The selected doses (0.5 mg and 1.0 mg sublingual; 1.0 mg IV) fall within approved adult therapeutic ranges, enabling robust yet ethically appropriate characterization in healthy volunteers while informing exposure–response modeling for antimuscarinic pharmacodynamics relevant to emergency care [4,5]. These anticipated benefits reflect interpretive synthesis of regulatory guidance with atropine's established clinical role.

Potential risks

Risks are primarily related to predictable antimuscarinic pharmacology of atropine and to study procedures.

- Pharmacologic risks: transient tachycardia, palpitations, increased blood pressure, dizziness, blurred vision/mydriasis, xerostomia, decreased sweating with heat intolerance, constipation, urinary hesitancy/retention, and, at higher exposures, agitation or confusion; rare serious events include arrhythmias or angle-closure glaucoma in susceptible individuals [4,5]. Ophthalmic formulations may cause mild local oral irritation or unpleasant taste; systemic toxicity from excipients at the administered micro-volumes is not expected [5].
- Procedural risks: IV cannulation and serial phlebotomy may cause pain, bruising, vasovagal reactions, infiltration, local infection, or phlebitis; total blood volume and sampling frequency are within accepted best-practice limits for adult research participants when performed by trained personnel [6]. Short nil-per-os windows and study confinement may contribute to transient discomfort.

In this healthy adult cohort without therapeutic intent, these foreseeable risks are time-limited, reversible in most cases, and well described in product labeling and clinical experience [4,5].

Risk minimization and management

Risk is minimized through scientifically justified eligibility criteria, standardized dosing conditions, continuous safety oversight, and adherence to Good Clinical Practice (GCP).

- Eligibility safeguards: exclusion of participants with cardiovascular disease, significant conduction abnormalities (including QTc thresholds), uncontrolled hypertension, angle-closure glaucoma or predisposing ocular history, gastrointestinal hypomotility disorders, prostatic hypertrophy/urinary retention, myasthenia gravis, clinically significant hepatic/renal impairment, and known atropine hypersensitivity aligns with labeled contraindications and precautions, reducing susceptibility to serious antimuscarinic events [4,5].
- Controlled dosing and sampling: single-dose administration per period with approximately 7-day washouts exceeds five half-lives, minimizing carryover [4]. Standardized sublingual technique and short nil-per-os intervals limit swallowing and absorption variability while reducing aspiration risk [1,2,5].
- Medical oversight: continuous or frequent vital sign and cardiac monitoring during the early postdose window, on-site advanced life support capability, and predefined management algorithms (e.g., evaluation for symptomatic tachyarrhythmias, urinary retention, or acute angle closure) enable prompt identification and treatment of adverse events per labeling recommendations [4,5]. Trained staff perform venipuncture using aseptic technique and apply WHO-recommended practices to minimize complications from blood collection [6].
- GCP compliance: conduct under ICH E6(R2), including independent ethics review, informed consent, qualified investigators, validated bioanalytical methods, and safety reporting, ensures systematic protection of participant rights, safety, and well-being [7].

These measures collectively reduce the probability and severity of anticipated events and ensure rapid intervention if they occur. This synthesis reflects expert interpretation anchored in the cited guidance.

Benefit–risk conclusion

Considering the public health need for rapidly deployable atropine in nerve agent incidents, the absence of definitive absolute bioavailability data for sublingual use of the widely available 1% ophthalmic solution, and regulatory expectations for IV-referenced characterization, the study's scientific and developmental value is substantial [1–3]. The investigational exposures are within approved adult therapeutic ranges, pharmacologic effects are well characterized and monitorable, and procedural risks are routine and mitigated by protocol controls, trained staff, and GCP adherence [4,5,7]. On balance, based on available evidence, the anticipated benefits of obtaining robust, regulatory-grade bioavailability and PK comparability data outweigh the foreseeable, manageable risks to participants in this healthy volunteer study.

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3 OBJECTIVES AND ENDPOINTS

3.1 Primary Objective and Endpoint

Primary objective

To characterize and compare the bioavailability and pharmacokinetic properties of atropine after single sublingual administration of 0.5 mg and 1.0 mg atropine sulfate ophthalmic solution USP 1% (test) relative to a 1.0 mg intravenous (IV) reference dose of atropine sulfate injection in healthy adults, enabling estimation of absolute bioavailability and assessment of the rate and extent of absorption using established regulatory pharmacokinetic endpoints and comparison methods [1,2].

Primary endpoints

Primary pharmacokinetic endpoints for plasma atropine are:

- Extent of exposure: area under the plasma concentration–time curve from time zero to the last quantifiable concentration (AUC0–t) and extrapolated to infinity (AUC0–∞) [1,2].

- Rate of absorption: maximum observed plasma concentration (Cmax) and time to Cmax (tmax) [1,2].
- Disposition parameters: terminal elimination half-life (t½), apparent clearance after extravascular dosing (CL/F), and apparent volume of distribution after extravascular dosing (Vd/F) [1].

Absolute bioavailability (F) for each sublingual dose will be derived by comparing AUC (dose-normalized where appropriate) following sublingual administration with AUC after the IV reference, consistent with FDA and EMA guidance for IV-referenced bioavailability assessments [1,2].

Endpoint evaluation and regulatory relevance

- Extent of absorption will be concluded primarily from AUC metrics (AUC0–t and AUC0–∞), which reflect the quantity of drug reaching the systemic circulation; comparison of sublingual versus IV AUC provides the basis for estimating absolute bioavailability and for determining exposure comparability across routes [1,2].
- Rate of absorption will be evaluated using Cmax and tmax; together these characterize the speed and magnitude of systemic entry via the sublingual route relative to IV, in line with regulatory constructs for assessing absorption rate differences between products and routes [1,2].
- Disposition parameters (t½, CL/F, Vd/F) contextualize absorption findings and support interpretation of exposure comparability by confirming similar post-absorption elimination kinetics across periods, as recommended for absolute bioavailability studies employing noncompartmental analysis [1].

For quantitative comparisons, AUC (AUC0–t and AUC0–∞) and Cmax will be natural log-transformed and analyzed in a mixed-effects model to obtain geometric least-squares mean ratios and 90% confidence intervals (CIs) for: low-dose sublingual versus IV, high-dose sublingual versus IV, and low-dose versus high-dose sublingual. Presentation of 90% CIs for exposure ratios follows established regulatory practice for pharmacokinetic comparability; conventional bioequivalence interpretive limits (80.00%–125.00%) provide context for judging closeness of exposure, recognizing this is a descriptive absolute bioavailability study rather than a formal bioequivalence trial [2,3]. Tmax will be summarized nonparametrically [2]. This analytic framework operationalizes accepted criteria for assessing the rate and extent of absorption and pharmacokinetic comparability in crossover bioavailability designs using an IV reference [1–3].

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3.2 Secondary Objectives and Endpoints

Secondary objectives

- To further characterize early absorption and distribution kinetics after sublingual dosing, including partial exposure in the first hour postdose, to contextualize the primary absolute bioavailability comparison and onset profile [1,2].

- To assess dose proportionality of exposure between 0.5 mg and 1.0 mg sublingual doses using established power-model methods, supporting interpretation of dose-related pharmacokinetics within the studied range [1,2].
- To estimate intra- and inter-subject variability for key exposure metrics (AUC, Cmax) across routes and doses in a crossover framework, with attention to absorption-related variability expected for sublingual delivery [2,3].
- To evaluate safety and tolerability of single-dose sublingual atropine (0.5 mg and 1.0 mg) compared with IV atropine (1.0 mg), including treatment-emergent adverse events (TEAEs), vital signs, ECG parameters, and participant-reported xerostomia, given dry mouth is a predictable antimuscarinic effect and a practical tolerability marker for sublingual use [4–6].

Secondary endpoints

Pharmacokinetic

- Early partial AUCs to characterize rate of systemic entry after sublingual dosing: pAUC0–15min, pAUC0–30min, and pAUC0–60min (linear-up/log-down), summarized and compared descriptively across treatments; selection of early windows aligns with regulatory guidance for rate-sensitive secondary metrics [1,2].
- Absorption lag time (tlag), if quantifiable, and early concentration-time profile features (e.g., time to first quantifiable concentration), summarized descriptively [1,2].
- Dose proportionality assessment for sublingual 0.5 mg vs 1.0 mg using the power model on ln(AUC0–t, AUC0–∞, Cmax) to estimate the slope and its confidence interval [1,2].
- Variability estimates: within-subject and between-subject coefficients of variation for AUC0–t, AUC0–∞, and Cmax by treatment [2].

Safety and tolerability

- Incidence, severity, and relatedness of TEAEs and SAEs from dosing through end of each period, coded by system organ class and preferred term; AE/SAE definitions will follow ICH standards [4].
- Vital signs (supine blood pressure and heart rate) change from baseline over at least 4 hours postdose; descriptive summaries and treatment comparisons emphasize early postdose intervals when antimuscarinic effects peak [5].
- 12-lead ECG parameters (PR, QRS, QTc using Fridericia's correction) change from baseline and categorical outlier summaries, with attention to predefined exclusion thresholds [4,5].
- Clinical laboratory tests (hematology, chemistry, urinalysis): shift and outlier analyses from screening/baseline to postdose assessments [4].
- Local tolerability of the oral mucosa after sublingual dosing (investigator inspection and participant report), summarized by treatment [1].
- Participant-rated xerostomia intensity using a numeric rating scale (0=no dryness, 10=worst possible dryness) at predose and every 10 minutes up to 60 minutes postdose, then hourly to 4 hours, summarized by treatment as change from baseline and area under the effect–time curve (AUEC0–60min); numeric rating scales are commonly employed to quantify acute mouth dryness and are suitable for frequent serial assessment [3,6].

Planned secondary analyses and their interpretive role

- For partial AUCs and early PK descriptors, log-transformed pAUCs will be analyzed in the same mixed-effects model structure used for primary AUC/Cmax to obtain geometric mean ratios and 90% confidence intervals, providing context on early exposure differences that may influence onset of effect; tmax and time to first quantifiable concentration will be summarized nonparametrically in line with regulatory guidance [1,2]. This supports interpretation of rate-of-absorption differences between sublingual and IV routes.
- Dose proportionality across 0.5 mg and 1.0 mg sublingual doses will be explored using a power model (ln[PK] = α + β·ln[dose] + random effects). Point estimates and 90% confidence intervals for β will be presented; β close to 1 indicates approximate proportionality. Results inform whether exposure scales predictably across the evaluated sublingual dose range [1,2].

- Variability: within-subject and between-subject variance components for ln(AUC) and ln(Cmax) will be estimated from the mixed model to quantify absorption-related variability with the sublingual route versus IV. Findings inform precision of the absolute bioavailability estimates and feasibility of future comparative studies [2,3].
- Safety/tolerability: TEAEs, vital signs, ECGs, and xerostomia will be summarized descriptively by treatment and period. For xerostomia, change-from-baseline profiles and AUEC0–60min will be compared using mixed models to relate early exposure to tolerability signals. As dry mouth is a predictable, mechanism-based effect of atropine, its time course provides supportive context for the PK findings and practical tolerability of sublingual administration [5,6].

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3.3 Exploratory Objectives and Endpoints

Exploratory objectives

- To explore additional characteristics of sublingual absorption using descriptive and model-based analyses (e.g., modeling-derived absorption rate constants, mean residence time, and shape descriptors of the early concentration–time curve), complementing primary noncompartmental endpoints [1,2,6].
- To investigate exposure–response relationships between plasma atropine concentrations and pharmacodynamic effects measurable in this study (heart rate, blood pressure, and participant-reported xerostomia), to inform onset and tolerability profiling for future dosing recommendations [3–5,7].
- To identify potential sources of inter-individual variability in rate and extent of exposure following sublingual dosing by evaluating covariate relationships (e.g., sex, age, body weight/BMI, sequence/period) within the crossover framework, recognizing that oral mucosal physiology can influence transmucosal absorption [2,3,6].
- To generate preliminary, hypothesis-forming evidence regarding whether sublingual disposition is elimination-limited (i.e., absence of flip–flop kinetics) through comparison of terminal slopes across routes and model-based sensitivity analyses [1,6].
- To assess qualitative concordance between early exposure metrics and the time course of xerostomia and heart rate changes as pragmatic, mechanism-based markers of antimuscarinic activity after sublingual administration [4,7].

Exploratory assessments are hypothesis-generating, not powered for confirmatory inference, and will be interpreted descriptively without multiplicity-controlled conclusions, consistent with good practice for exploratory analyses in early clinical pharmacology [1,5].

Exploratory endpoints

Pharmacokinetic characterization

- Model-derived parameters from structural compartmental analyses for each treatment: first-order absorption rate constant (ka) for sublingual dosing, model-based Cmax and tmax, mean residence time (MRT), and model-estimated terminal elimination rate constant (λz) compared across routes to probe absorption versus disposition control [1,6].
- Additional descriptive PK shape features for sublingual dosing: time to first quantifiable concentration, early concentration slope from 2–10 minutes, and partial exposure fractions (pAUC0–15min/AUC0–∞ and pAUC0–30min/AUC0–∞) beyond secondary analyses, summarized to characterize onset dynamics [1,2].

Exposure–response and tolerability linkage

- Relationship between plasma atropine concentrations and change from baseline in heart rate using prespecified time-matched summaries and exploratory Emax or indirect-response models; parameters (e.g., EC50, Emax) to be estimated with uncertainty intervals where feasible [4,7].
- Relationship between exposure metrics (AUC0–60min, Cmax, and model-derived ka) and xerostomia intensity/time course (change-from-baseline profiles and AUEC0–60min), summarized descriptively and via exploratory mixed-effects models [4,7].
- Qualitative concordance of early exposure (e.g., pAUC0–15min, pAUC0–30min) with onset of heart rate increase and xerostomia, summarized by treatment to inform future onset-targeted dosing [1,2,4].

Variability and covariate exploration

- Intra- and inter-individual variability components from population or mixed-effects models for AUC and Cmax, contrasted between sublingual and IV routes to contextualize absorption-related variability [2,6].
- Covariate screening on sublingual exposure metrics (AUC, Cmax, ka) for demographic and design factors (sex, age, body weight/BMI, sequence/period), reported as effect estimates with confidence intervals and graphical diagnostics to generate hypotheses about sources of variability in transmucosal absorption [2,3,6].

Interpretive note: These exploratory endpoints are intended to generate hypotheses and supportive insights for future studies, formulation optimization, and methodological refinement; they are not intended to support formal inferential conclusions or labeling claims [1,5].

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4 STUDY DESIGN

4.1 Overall Design Description

Overview of design

This is a randomized, open-label, single-dose, three-sequence, three-period crossover bioavailability study conducted in healthy adults to characterize atropine exposure after sublingual administration of atropine sulfate ophthalmic solution USP 1% at 0.5 mg and 1.0 mg compared with an intravenous (IV) reference dose of 1.0 mg atropine sulfate injection. The design enables within-participant comparisons of the rate and extent of absorption across routes and doses, consistent with regulatory guidance for absolute bioavailability assessments using an IV reference and crossover configuration to control inter-individual variability [1,2]. Primary pharmacokinetic endpoints (AUC0–t, AUC0–∞, Cmax, tmax) and disposition parameters are obtained following each treatment; treatment comparisons use log-transformed AUC and Cmax with mixed-effects modeling to derive geometric mean ratios and 90% confidence intervals, in accordance with established statistical approaches for pharmacokinetic comparability in crossover studies [1–3].

Study configuration and treatment allocation

Participants are randomized in a 1:1:1 ratio to one of three treatment sequences. Each participant receives three single doses, one per period, separated by washouts. Open-label administration is employed due to inherent differences in route and dose presentation.

Sequence	Period 1	Period 2	Period 3
A	0.5 mg sublingual (SL)	1.0 mg SL	1.0 mg IV
B	1.0 mg SL	1.0 mg IV	0.5 mg SL
C	1.0 mg IV	0.5 mg SL	1.0 mg SL

Randomization is implemented using a computer-generated schedule with balanced blocks to maintain approximately equal allocation to each sequence; assignment occurs at check-in for Period 1. The crossover, sequence-balanced structure mitigates period and sequence effects and supports unbiased within-participant treatment contrasts, consistent with bioavailability/bioequivalence methodological guidance [2,3].

Study periods, washout, and participant flow

- Screening: up to approximately 28 days before first dosing to confirm eligibility by medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratory tests, conducted under ICH Good Clinical Practice (GCP) [4].
- Treatment periods: Dosing on Days 1, 8, and 15. Each period includes predose assessments, single-dose administration, intensive pharmacokinetic sampling through 8 hours postdose, and safety monitoring for at least 4 hours postdose (vital signs and ECGs as indicated).
- Washout: Approximately 7 days between doses, exceeding five terminal half-lives for atropine in healthy adults to prevent pharmacokinetic carryover; atropine exhibits a terminal half-life on the order of a few hours in adults, making a 7-day interval conservative and consistent with regulatory expectations for washouts greater than 5 half-lives in crossover designs [2,5].
- End-of-study: A safety assessment after completion of Period 3 or upon early discontinuation, per GCP [4].

Dosing conditions and administration procedures

All doses are administered under fasting conditions with no food or drink from 1 hour before until 1 hour after dosing; the first postdose meal is provided no earlier than 4 hours postdose. Alcohol is prohibited from 24 hours before each dosing until completion of the final pharmacokinetic sampling. These conditions align with regulatory recommendations to minimize food effects and variability during bioavailability and bioequivalence assessments [1,2].

- Sublingual dosing: Commercial atropine sulfate ophthalmic solution USP 1% (10 mg/mL; NDA 206289) is administered as drops placed under the tongue at 0.5 mg (50 µL) or 1.0 mg (100 µL). Participants are instructed to avoid swallowing and to minimize orofacial movement for several minutes postdose to support transmucosal exposure, consistent with standard practice for buccal/sublingual administration under fasting conditions [1,2].
- Intravenous dosing: Commercial atropine sulfate injection 0.4 mg/mL (NDA 209260) is administered as a 1.0 mg (2.5 mL) IV dose via an indwelling cannula under continuous clinical supervision, consistent with labeling and clinical practice for parenteral atropine [5].

Pharmacokinetic and safety assessments

- Pharmacokinetics: Blood samples for plasma atropine are collected predose and at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes, and at 2, 4, 6, and 8 hours postdose in each period. This schedule is designed to capture the absorption phase after sublingual dosing, early distribution after IV dosing, and sufficient terminal data to characterize elimination and estimate AUC0–∞ and t½ with noncompartmental methods, as recommended for absolute bioavailability studies [1,2].
- Safety/tolerability: Treatment-emergent adverse events are collected from dosing through end-of-period assessments; vital signs (supine blood pressure and heart rate) are measured repeatedly for at least 4 hours postdose; 12-lead ECGs and clinical laboratory tests are obtained per protocol. Xerostomia is recorded using a numeric rating scale at prespecified frequent intervals during the first hour, reflecting anticipated antimuscarinic effects and serving as a tolerability marker, with oversight and procedures compliant with ICH GCP [2,4,5].

Population, eligibility, and sample size

Healthy adult males and non-pregnant, non-breastfeeding females aged 18–55 years are eligible based on screening evaluations indicating good health. Key exclusions include clinically significant cardiovascular disease or arrhythmias, QTc prolongation (≥450 ms for males, ≥470 ms for females), uncontrolled hypertension (>140/90 mmHg), glaucoma or history of ocular surgery, gastrointestinal motility disorders, urinary retention or prostatic hypertrophy, pre-existing xerostomia, myasthenia gravis, thyroid disease, hepatic or renal impairment, positive tests for HIV/HBV/HCV, recent prescription drug use (within 30 days), substance abuse, or known hypersensitivity to atropine; women of childbearing potential must use highly effective contraception. These criteria are appropriate for minimizing risk from antimuscarinic effects and for reducing confounding factors in healthy volunteer pharmacokinetic studies conducted to GCP standards [2,4,5].

Approximately 15 participants will be enrolled to achieve about 12 evaluable completers (approximately 4 per sequence), allowing for anticipated attrition. As a descriptive absolute bioavailability study, the sample size is based on operational feasibility and the within-subject precision afforded by the 3-period crossover rather than on a formal hypothesis-testing calculation, which is consistent with regulatory guidance for early-phase bioavailability designs aimed at estimation of exposure metrics and their variability [1–3].

Analysis framework and regulatory alignment

Within-participant comparisons across treatments will be performed on ln-transformed AUC0–t, AUC0–∞, and Cmax using a mixed-effects model including fixed effects for treatment, period, and sequence and a random effect for participant within sequence; geometric least-squares mean ratios

and 90% confidence intervals will be presented for prespecified contrasts. Tmax will be summarized nonparametrically. This approach follows FDA and EMA guidance for crossover bioavailability/bioequivalence analyses and standard statistical principles for estimating treatment effects and associated uncertainty using confidence intervals rather than formal equivalence testing in a descriptive absolute bioavailability context [1–3]. The overall configuration—randomized, open-label, multiple-period crossover with adequate washouts under fasting conditions and intensive pharmacokinetic sampling—conforms to applicable bioavailability and bioequivalence guidance and provides a scientifically robust and operationally efficient basis for estimating the absolute bioavailability of sublingual atropine relative to IV reference [1,2].

References

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4.2 Scientific Rationale for Study Design

Overall design choice and within-subject comparison

A randomized, single-dose, three-period crossover with an IV reference is the established design to estimate absolute bioavailability (F) and to compare the rate and extent of absorption across routes while minimizing between-subject variability through within-subject contrasts [1,2]. Regulatory guidance explicitly recommends inclusion of an intravenous reference to derive F and endorses crossover configurations for BA/BE assessments when feasible, with analysis based on log-transformed AUC and Cmax using mixed-effects models to obtain geometric mean ratios and confidence intervals [1–3]. Open-label administration is appropriate because route and device/formulation presentations are inherently distinguishable and blinding would not mitigate bias for objective PK endpoints derived from bioanalytical measurements [2].

The three-sequence structure balances period and sequence effects and enables unbiased estimation of treatment contrasts for 0.5 mg sublingual (SL) vs IV, 1.0 mg SL vs IV, and 0.5 vs 1.0 mg SL within subjects, consistent with EMA and FDA recommendations to control inter-individual variability and to assess rate and extent of absorption through crossover methodology [1–3].

Dose selection and clinical context

Single doses of 0.5 mg and 1.0 mg SL are bracketed around the labeled adult IV dose range used clinically for bradycardia (0.5–1.0 mg IV), providing a clinically relevant exposure context while limiting anticholinergic burden in healthy volunteers [4]. A 1.0 mg IV reference enables dose-normalized comparisons and direct estimation of absolute F in line with FDA BA guidance for IV-referenced studies [1]. Evaluating two SL doses allows assessment of dose–exposure relationships over a pragmatic range for emergency, rapidly deployable countermeasure use, while preserving safety in a Phase 1 setting [1,4].

Dosing conditions and control of extrinsic variability

Fasting conditions are specified to reduce variability from salivary flow, swallowing, and gastrointestinal transit that can alter transmucosal and oral contributions to exposure. FDA and EMA recommend standardized fasting administration for BA/BE assessments of immediate-release products to minimize confounding food effects and to maximize assay sensitivity for detecting absorption differences [1,2]. Avoidance of fluids around dosing, deferred meals, and prohibition of alcohol are consistent with these recommendations and with best practices for buccal/sublingual delivery, where residence time and saliva dynamics materially influence absorption and variability [1–3,5].

Washout duration and carryover control

A washout of approximately 7 days exceeds five terminal half-lives of atropine in healthy adults (terminal t½ on the order of hours), thereby satisfying regulatory expectations to preclude pharmacokinetic carryover in crossover designs [2,4]. This interval is conservative relative to known atropine disposition following IV administration and provides ample time for complete elimination between periods, supporting unbiased within-subject comparisons [2,4].

PK sampling schedule and endpoint suitability

Intensive early sampling (2–60 minutes) captures the absorption phase after SL dosing and the rapid distribution following IV administration, while later samples through 8 hours support robust estimation of AUC0–∞ and terminal half-life. FDA and EMA recommend sampling dense enough around expected Cmax and sufficient in the terminal phase to enable reliable noncompartmental estimation of AUC and t½, which are the primary endpoints for assessing extent and rate of absorption and for deriving absolute F versus IV [1,2]. The prespecified AUC0–t, AUC0–∞, Cmax, and tmax align with these guidances and are standard for BA determinations [1–3].

Expected variability, precision requirements, and sample size

Sublingual and broader buccal delivery can exhibit higher absorption-related variability due to mucosal permeability, saliva-mediated dilution, and inadvertent swallowing, whereas IV administration has low within-subject variability in exposure by design [2,5]. A crossover design therefore materially improves precision for exposure ratios by leveraging within-subject contrasts. In early absolute BA studies focused on estimation, FDA and EMA allow descriptive analyses with confidence intervals rather than formal equivalence testing, and sample sizes are commonly based on operational feasibility and expected within-subject variance, especially when safety constraints limit dose escalation in healthy volunteers [1–3]. Enrolling approximately 15 participants to achieve about 12 evaluable completers in a three-period crossover provides adequate precision for geometric mean ratio estimates and 90% confidence intervals for AUC and Cmax to meet the study's estimation objectives, while enabling characterization of intra- and inter-subject variability to inform future development [1–3,5]. This precision-focused, estimation-oriented approach is consistent with ICH E9 statistical principles for early-phase trials emphasizing estimation and uncertainty quantification over hypothesis testing [6].

Alignment with objectives and statistical analysis

The design directly supports the primary objective to characterize and compare the bioavailability and PK of atropine after SL administration relative to IV by enabling within-subject estimation of geometric mean ratios and 90% confidence intervals for AUC and Cmax, with tmax summarized nonparametrically per regulatory norms [1–3]. Secondary and exploratory objectives (dose proportionality, early partial AUCs, and variability estimation) are accommodated by the two SL dose levels and dense early sampling, and the crossover structure yields variance components needed for dose proportionality modeling and for quantifying within-subject variability, as recommended for methodological completeness in BA assessments [1–3]. Collectively, these elements ensure that the study structure, procedures, and analysis plan are scientifically and regulatorily coherent with the intended absolute bioavailability and comparability objectives.

Ethical and safety considerations in healthy volunteers

Use of healthy adults with exclusion of conditions that elevate risk from antimuscarinic effects, combined with single-dose exposures within the approved IV therapeutic range, aligns with GCP and labeling-based risk minimization for atropine. Monitoring of heart rate and blood pressure conforms to known pharmacology and labeled effects, supporting participant safety while permitting acquisition of objective PK endpoints under controlled conditions [1,2,4].

Uncertainty and interpretive considerations

As is typical for transmucosal products, some variability in SL absorption is anticipated; the crossover design, fasting conditions, and standardized administration procedures are expected to mitigate—but not eliminate—this uncertainty. Interpretive inferences will be estimation-focused, with 90% confidence intervals contextualized against conventional comparability ranges without formal equivalence claims, consistent with current regulatory guidance for absolute BA studies [1–3]. This reflects consensus-based reasoning integrating guidance and route-specific considerations [1–3,5,6].

References

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4.3 Justification for Dose and Formulations

Rationale for selected dose levels

The study evaluates single sublingual doses of 0.5 mg and 1.0 mg atropine sulfate against a 1.0 mg intravenous (IV) reference to characterize absolute bioavailability and the rate/extent of absorption within a therapeutically relevant range. Adult IV atropine dosing for symptomatic bradycardia begins at 0.5 mg with 1.0 mg commonly used, anchoring the clinical relevance of the selected range and supporting safety in healthy volunteers [1,3]. Regulatory guidance for bioavailability studies endorses dose selection within a pharmacokinetically informative and clinically relevant span, particularly when assessing linearity and enabling precise within-subject contrasts to an IV reference [1,2]. The two sublingual (SL) dose levels permit evaluation of dose–exposure relationships while constraining antimuscarinic burden in a Phase 1 setting [1–3]. The 1.0 mg IV reference provides a robust denominator for absolute bioavailability estimation and dose-normalized comparisons in line with FDA and EMA recommendations [1,2].

Therapeutic and pharmacokinetic considerations indicate that single 0.5–1.0 mg doses are expected to produce measurable plasma concentrations with predictable antimuscarinic pharmacology (e.g., heart rate increase, xerostomia) under close monitoring, consistent with labeled effects and the known clinical profile of atropine [1,3]. The elimination half-life of atropine in adults is on the order of hours per product labeling, supporting an 8-hour sampling window to estimate AUC0–∞ and an

approximate 7-day washout exceeding five half-lives for carryover control [3].

Pharmaceutical forms, routes, and administration methods

- Test product: Commercial atropine sulfate ophthalmic solution USP 1% (10 mg/mL; NDA 206289) administered sublingually as 0.5 mg (50 µL) and 1.0 mg (100 µL) unit doses placed under the tongue using calibrated drops. Although labeled for ophthalmic use, the sterile aqueous solution provides an immediate-release presentation suitable for transmucosal application when administered under controlled, fasting conditions [2,4]. Small-volume administration and instructions to avoid swallowing optimize residence time and minimize gastrointestinal contribution, consistent with established principles for buccal/sublingual delivery [1,2,5].
- Reference product: Commercial atropine sulfate injection 0.4 mg/mL (NDA 209260), administered as 1.0 mg (2.5 mL) IV via indwelling cannula, consistent with labeling and clinical practice, to provide the reference systemic input for absolute bioavailability estimation [1,3].

Dosing conditions (fasting state, fluid restrictions around dosing) and standardized administration techniques are aligned across periods to minimize extrinsic variability and maximize assay sensitivity for detecting absorption differences, consistent with bioavailability/bioequivalence guidance [1,2].

Formulation characteristics and justification of comparability

Both the test and reference products contain the same active moiety (atropine sulfate) in aqueous solution dosage forms that release drug immediately without a controlled-release mechanism. This supports scientific comparability of systemic exposure profiles when differences are attributable to route of administration rather than formulation release characteristics, which is appropriate for absolute bioavailability assessments [1,2]. The selected sublingual volumes (50–100 µL) are within practical limits for mucosal dosing, reducing involuntary swallowing and facilitating rapid transmucosal uptake where permeability and local residence time govern early systemic entry [2,5]. The ophthalmic solution's established concentration (1% = 10 mg/mL) enables accurate, low-volume dosing under direct observation, improving dose precision for crossover comparisons [4].

Support from nonclinical/clinical knowledge and prior human experience

Transmucosal absorption across the oral mucosa is well-established for small, lipophilic or ionizable drugs delivered as solutions, with rapid onset possible when residence time is maintained and volumes are minimized [5]. Clinical experience with sublingual administration of atropine ophthalmic drops has demonstrated systemic antimuscarinic effects (e.g., xerostomia) at low doses, supporting feasibility and tolerability of sublingual delivery in adults under monitored conditions [6]. Together with labeled IV pharmacology and PK (rapid distribution, measurable plasma concentrations within minutes, elimination over hours), these data support that 0.5–1.0 mg SL doses are pharmacokinetically informative and safe for an estimation-focused absolute bioavailability study in healthy volunteers [1–3,6]. This interpretive synthesis integrates guidance-based principles, labeled pharmacology, and route-specific literature to justify the selected dosing and formulations for the intended objectives.

Alignment with bioavailability assessment objectives

- The 1.0 mg IV reference allows direct estimation of absolute bioavailability (F) for each SL dose via AUC comparisons, as recommended by FDA and EMA [1,2].
- Two SL dose levels within the labeled IV therapeutic range support characterization of exposure proportionality and early absorption metrics without excessive anticholinergic risk in healthy participants [1–3].
- Immediate-release aqueous solutions administered under standardized fasting conditions ensure that observed differences in exposure reflect route-dependent absorption kinetics

rather than formulation-controlled release, meeting methodological expectations for bioavailability evaluation [1,2].

References

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4.4 End of Study Definition

Definition of end of study

The end of the study is defined as the date on which the last enrolled participant completes the end-of-study (EOS) visit, operationally equivalent to Last Participant Last Visit (LPLV). For participants who complete all three periods, the EOS visit occurs after completion of Period 3 assessments; for participants who discontinue early, the EOS visit is the Early Termination (ET) visit conducted as soon as feasible after discontinuation. Adverse event (AE) and serious adverse event (SAE) collection and safety monitoring continue through the EOS/ET visit, consistent with Good Clinical Practice, with SAEs and clinically significant AEs followed to resolution or stabilization even if this extends beyond the EOS/ET visit [1–4].

Scope of data collection and safety monitoring until EOS

- All protocol-specified data, including pharmacokinetic samples and safety/tolerability assessments, are collected through the participant's EOS/ET visit. AE/SAE reporting requirements apply from first study intervention through EOS/ET, with expedited reporting for SAEs per applicable regulations and guidance [1,2,4].
- Unresolved or clinically significant findings at EOS/ET (e.g., ongoing AE, abnormal laboratory or ECG result deemed clinically significant) are followed until resolution, return to baseline, or stabilization, as required by ICH GCP and safety reporting guidance [1,4].

Early withdrawals, discontinuations, and protocol deviations

- Participants who withdraw consent, are withdrawn by the investigator, or discontinue study treatment for any reason will be asked to complete an ET visit comprising the EOS safety assessments and, if feasible, any outstanding protocol-specified procedures; data collected

up to ET will be included in analyses per the statistical analysis plan. Such early discontinuations do not alter the study-level EOS definition (LPLV) [1–3].

- Protocol deviations (major or minor) do not modify the EOS definition. Participants with major deviations may be excluded from per-protocol analyses but remain in safety and other prespecified analysis sets as defined elsewhere in the protocol; all safety data through EOS/ET are retained [1,3].

Post-EOS closeout

Administrative closeout activities (e.g., data verification, database lock, final analyses, clinical study report) occur after the EOS date. Safety information meeting expedited reporting criteria identified after EOS but relating to on-study exposure will be reported in accordance with applicable regulations and guidance [2,4].

References

1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). E6(R2) Guideline for Good Clinical Practice. Geneva: ICH; 2016. Available from: https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf
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5 STUDY POPULATION

5.1 Inclusion Criteria

Eligibility criteria

Participants must meet all of the following criteria to be enrolled. Criteria are designed to protect participant safety and ensure interpretable pharmacokinetic comparisons in healthy adults consistent with regulatory guidance for bioavailability studies.

- Signed and dated informed consent obtained before any study-specific procedures, with capacity and willingness to comply with all protocol requirements, confinement, fasting, alcohol restrictions, dosing, intravenous cannulation, intensive pharmacokinetic sampling, and safety monitoring, in accordance with ICH Good Clinical Practice [1].
- Male or female, 18 to 55 years of age (inclusive) at screening; healthy volunteer status appropriate for bioavailability/bioequivalence assessments in crossover design studies [2,3].
- General good health as determined by the investigator based on:

- Medical history and complete physical examination without clinically significant abnormalities [1].
- Vital signs within normal ranges or not clinically significant in the opinion of the investigator.
- 12-lead ECG at screening and predose showing normal morphology without clinically significant abnormalities; corrected QT interval (QTc, Fridericia) <450 ms for males and <470 ms for females [3,5].
- Clinical laboratory evaluations (hematology, serum chemistry, urinalysis) within normal limits or deviations assessed as not clinically significant.

- Body weight ≥50 kg for males and ≥45 kg for females, and overall body habitus judged by the investigator to be compatible with safe phlebotomy and intravenous dosing in a Phase 1 setting [1].

- Negative tests for human immunodeficiency virus (HIV-1/2), hepatitis B surface antigen (HBsAg), and hepatitis C virus antibody (anti-HCV) at screening.
- Ability and commitment to adhere to protocol-specified dietary and behavioral restrictions, including fasting from 1 hour before until 1 hour after each dose, standardized timing of the first meal ≥4 hours postdose, and abstinence from alcohol from 24 hours before each dosing through completion of final pharmacokinetic sampling, consistent with bioavailability guidance to minimize variability [2,3].
- Women of nonchildbearing potential (postmenopausal with ≥12 months of spontaneous amenorrhea or permanently sterile via bilateral oophorectomy, hysterectomy, or bilateral salpingectomy) are eligible; documentation per site standard is required [4].
- Women of childbearing potential (WOCBP) are eligible if they have:

- A negative serum pregnancy test at screening and a negative urine pregnancy test before each dosing period, and
- Agreed to use at least one highly effective method of contraception (failure rate <1% per year when used consistently and correctly; e.g., combined hormonal contraception associated with inhibition of ovulation, progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, or sexual abstinence as the participant's usual and preferred lifestyle) from at least 14 days before first dosing until 30 days after last dosing [4].

- Sexually active male participants agree to use a condom (with spermicide where locally available) during intercourse with a WOCBP partner from first dosing until 30 days after last dosing and agree to refrain from sperm donation during this period, in line with contraception recommendations for early-phase trials [4].
- Willingness to abstain from prescription medications, over-the-counter drugs, herbal products, and dietary supplements per protocol and to avoid vaccines and procedures that could interfere with safety or pharmacokinetic assessments for the duration of study participation, unless approved by the investigator as not clinically relevant [2,3].

References

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5.2 Exclusion Criteria

Exclusion criteria are applied to protect participant safety and preserve the interpretability of pharmacokinetic outcomes in this absolute bioavailability study of atropine across sublingual and intravenous routes [1,2,4].

- Any clinically significant disease or condition that, in the investigator's judgment, could increase risk from antimuscarinic exposure or confound pharmacokinetics or safety assessments, including abnormal findings on medical history, physical examination, vital signs, 12-lead ECG, or clinical laboratory tests [1,2,4].

- Known hypersensitivity or contraindication to atropine, belladonna alkaloids, or any component of the test or reference products; history of severe idiosyncratic reaction to antimuscarinic agents [3].
- Cardiovascular conditions that increase risk with atropine: clinically significant structural heart disease; symptomatic coronary artery disease; uncontrolled hypertension (screening or predose BP >140/90 mmHg); clinically significant arrhythmia or conduction disorder; resting sinus tachycardia deemed clinically significant; QTcF ≥450 ms (males) or ≥470 ms (females); or use of implanted pacemaker/ICD [2,3].
- Ophthalmic conditions contraindicated with antimuscarinics or raising safety concerns: narrow-angle/angle-closure glaucoma or history of elevated intraocular pressure; recent or planned ocular surgery where antimuscarinic effects are undesirable [3].
- Gastrointestinal or genitourinary conditions with risk of exacerbation by antimuscarinics: obstructive uropathy or significant benign prostatic hypertrophy with urinary retention; gastrointestinal obstructive disorders, paralytic ileus, severe ulcerative colitis or toxic megacolon; clinically significant gastroparesis or other motility disorders; pre-existing severe xerostomia that could interfere with sublingual absorption assessments [2,3,5].
- Neuromuscular or neurologic conditions adversely affected by atropine, including myasthenia gravis (unless for anticholinesterase poisoning, which does not apply in this healthy volunteer study) [3].
- Endocrine/hepatic/renal disorders: uncontrolled thyroid disease; moderate-to-severe hepatic impairment or renal impairment deemed clinically significant for study participation or interpretation of PK [1,2,3].
- Positive screening tests for HIV-1/2, hepatitis B surface antigen, or hepatitis C antibody [1,2].
- Pregnant or breastfeeding women; women of childbearing potential not using highly effective contraception as specified in the protocol, or with a positive pregnancy test at screening or predose in any period [1,6].
- Use of prescription medications within 30 days before first dosing or planned use during the study, unless approved by the investigator as not clinically relevant; use of over-the-counter medications, herbal products, or dietary supplements within 14 days (7 days for simple analgesics without anticholinergic properties) before first dosing or during the study, unless approved by the investigator [1,2].
- Use within 30 days before first dosing or planned use during the study of drugs known to meaningfully affect heart rate/conduction or cholinergic tone/salivation that could confound PK or safety readouts, including but not limited to: cholinesterase inhibitors or cholinomimetics; anticholinergics (e.g., tricyclic antidepressants with antimuscarinic effects, first-generation antihistamines, antipsychotics with anticholinergic properties); beta-blockers, non-dihydropyridine calcium channel blockers, Class I/III antiarrhythmics; decongestants with sympathomimetic effects; or other agents judged likely to interfere with absorption, distribution, metabolism, or elimination of atropine or with study endpoints [1–3].
- Use of nicotine-containing products (including smoking, vaping, patches, gum) within 30 days before first dosing or inability to abstain for the duration of each confinement period, due to effects on salivary flow and oral mucosal physiology that may alter sublingual absorption [2,5].
- Consumption of alcohol within 24 hours before any dosing or inability to abstain from alcohol through completion of the last pharmacokinetic sample, as per protocol restrictions; history of alcohol or drug abuse within the past 12 months or positive drug/alcohol screen at screening or check-in [1,2,4].
- Participation in another interventional clinical study or receipt of any investigational product within 30 days or 5 elimination half-lives (whichever is longer) before first dosing, or planned participation during this study [1,2].
- Significant blood loss (e.g., ≥450 mL whole blood) within 56 days, plasma donation within 14 days, or red blood cell donation within 8 weeks before first dosing, or plans to donate blood/plasma during the study, if deemed to compromise safety or data integrity [1,4].
- Any acute illness, infection, fever, or clinically significant inflammation within 7 days before dosing, or positive viral screening at admission per site standard, that could affect safety or PK assessments [1,2].
- Known bleeding diathesis, clinically significant anemia or coagulopathy, or ongoing anticoagulant/antiplatelet therapy that would increase risk with phlebotomy or intravenous cannulation [1,4].
- Inability to comply with protocol-mandated fasting, fluid restrictions, standardized meals, confinement, serial PK sampling, safety monitoring, or contraception requirements; or any

condition (including anticipated poor adherence) likely to result in protocol deviations affecting data integrity [1,2,4].

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5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Fasting status, fluids, and timing of meals

- Each dosing period will be conducted under an overnight fast of at least 10 hours. Only water is permitted during the fast, with up to 240 mL allowed within 1 hour predose; no water is permitted from 1 hour predose until 1 hour postdose to minimize variability in absorption, consistent with bioavailability/bioequivalence guidance [1,2].
- No food or non-water beverages are allowed from 1 hour predose until 1 hour postdose. The first standardized meal will be provided no earlier than 4 hours postdose; subsequent meals and snacks will be standardized in timing and composition across periods to reduce within-subject variability in pharmacokinetics [1,2].
- Standardized postdose meals will be prepared by the unit's metabolic kitchen using consistent menus across periods. Lunch (first meal ≥4 hours postdose) will target approximately 600–800 kcal with ~20–30% of total energy from fat; beverages will exclude caffeine and alcohol; no grapefruit/Seville orange components will be used [1,2].

Fed-condition dosing (not planned)

- This study is designed and will be conducted under fasted conditions. If a fed-condition period is introduced by protocol amendment, dosing will follow a standardized high-fat, high-calorie breakfast (approximately 800–1000 kcal, ~50% of total calories from fat) with dosing 30 minutes after the start of the meal, in accordance with FDA food-effect guidance; all other meals and beverage restrictions will remain standardized [3].

Restrictions on foods, beverages, and oral products

- Alcohol is prohibited from 24 hours before each dosing through collection of the final pharmacokinetic sample of the corresponding period [1].
- Caffeine/xanthine-containing products (e.g., coffee, tea, cola, energy drinks, chocolate) are prohibited from 24 hours before dosing until after completion of the last pharmacokinetic sample of the period to avoid effects on cardiovascular physiology that could confound safety assessments and variability [2].
- Grapefruit or Seville orange (fruit or juice) and other grapefruit-related citrus products are prohibited from 72 hours before dosing until completion of the last pharmacokinetic sample of the period due to potential modulation of intestinal drug-metabolizing enzymes/transporters, per bioequivalence guidance [2].
- Charcoal-broiled meats are prohibited from 24 hours before dosing until completion of the last pharmacokinetic sample of the period to avoid diet-induced modulation of drug-metabolizing enzymes [2].
- To protect the integrity of sublingual absorption, participants must refrain from chewing gum, sucking lozenges/hard candy, using mouthwashes, or consuming strongly acidic or basic beverages that may alter salivary pH and flow from 12 hours before dosing until at least 1 hour postdose; such factors are known to influence transmucosal drug absorption and should be standardized [4]. These restrictions are in addition to study-wide prohibitions on nicotine and herbal products specified elsewhere in the protocol.

Dietary compliance monitoring and documentation

- During in-clinic confinement, fasting status, permitted water intake, and consumption of all standardized meals/snacks will be directly observed by study staff and documented in source records and meal accountability logs. Any deviations will be recorded as protocol deviations with timing and details [1,5].
- Participants will be reminded of preadmission dietary restrictions (alcohol, caffeine, grapefruit/Seville orange, charcoal-broiled meat) and will provide a predose affirmation; targeted screening (e.g., alcohol breath test) may be performed per site SOPs. Noncompliance may lead to postponement of dosing or exclusion from per-protocol pharmacokinetic analyses as defined in the statistical analysis plan [1,2,5].

Emesis and events compromising pharmacokinetic interpretability

- The exact time of any emesis will be recorded. Consistent with bioequivalence guidance, pharmacokinetic data from a period with vomiting occurring at or before twice the median tmax for the treatment may be excluded from the primary pharmacokinetic analysis; later emesis will be handled per the statistical analysis plan. Decisions will be documented prior to unblinded analysis of pharmacokinetic data [2].
- If spitting, excessive salivation, or swallowing difficulties occur within the first hour postdose that could compromise sublingual absorption, the event and timing will be documented; these periods may be flagged for sensitivity analyses per the statistical analysis plan. This reflects protocol-specific interpretive judgment informed by transmucosal pharmacology principles [2,4].

References

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5.3.2 Alcohol, Caffeine, Tobacco, and Drug Use

Prohibitions and washout requirements

To minimize pharmacokinetic (PK) variability and ensure participant safety in this crossover absolute bioavailability study of atropine, the following restrictions apply across all screening, washout, and confinement periods:

- Alcohol: Prohibited from 24 hours before each dosing until collection of the final PK sample for that period; participants must remain abstinent during in-clinic confinement. Alcohol may increase cardiovascular variability and adverse event risk and is discouraged during washout intervals in bioavailability studies [1,2].
- Caffeine and other xanthines (coffee, tea, cola/energy drinks, chocolate, yerba mate, theophylline-containing products): Prohibited from 24 hours before dosing until completion of the final PK sample for that period to avoid confounding of heart rate/blood pressure and related safety assessments [2,10].
- Nicotine and tobacco products (combustible cigarettes, cigars, smokeless tobacco, e-cigarettes/vapes) and nicotine replacement therapy (gum, lozenges, patches, inhalers): Prohibited from 30 days before first dosing through end of participation due to effects on oral/salivary physiology relevant to sublingual absorption and potential cardiovascular confounding; environmental exposure should be minimized [2,4,14].
- Cannabis and cannabinoid-containing products (including THC- or CBD-containing vaping products, edibles, oils, and topicals): Prohibited from 30 days before first dosing through end of participation because cannabinoids can inhibit or induce drug-metabolizing enzymes/transporters and affect cardiovascular function; CBD in particular is a known inhibitor of several CYP isoenzymes [2,9].
- Recreational or illicit substances (e.g., cocaine, amphetamines, methamphetamine/MDMA, phencyclidine, opioids without prescription, synthetic cannabinoids, hallucinogens): Prohibited from screening through end of participation; use is incompatible with healthy volunteer status and may alter PK or safety [1,2,5].
- Prescription, over-the-counter (OTC), herbal, and dietary supplements:

  - Strong or moderate CYP/P-gp/OATP inducers (e.g., rifampin, carbamazepine, phenytoin, phenobarbital, primidone, St. John’s wort): Prohibited within 28 days before first dosing and throughout the study due to persistent induction after discontinuation; this conservative washout reflects interpretive application of drug–drug interaction (DDI) guidance to early-phase PK studies [2,8,11].
  - Strong or moderate CYP/P-gp/OATP inhibitors (e.g., clarithromycin, itraconazole, posaconazole, verapamil/diltiazem, ritonavir/cobicistat, grapefruit/Seville orange products): Prohibited within 14 days before first dosing and throughout the study to reduce absorption and disposition variability; food-based restrictions are specified separately in dietary guidance [2,7,11].
  - Anticholinergic or cholinomimetic agents and drugs that meaningfully affect heart rate or atrioventricular conduction (e.g., tricyclics with antimuscarinic effects, first-generation antihistamines, antipsychotics with anticholinergic properties, cholinesterase inhibitors, beta-blockers, certain antiarrhythmics): Prohibited within 30 days before first dosing and throughout the study to prevent pharmacodynamic confounding with atropine [1,2].
  - Other OTC/herbal/dietary products with uncertain DDI potential (including concentrated polyphenol, kava, kratom, ginkgo, ginseng, milk thistle, turmeric/curcumin, resveratrol, high-dose biotin): Prohibited within 14 days before first dosing and throughout the study unless explicitly approved by

the investigator as not clinically relevant to PK or safety, consistent with bioavailability guidance [1,2].

These restrictions extend through each approximately 7-day interperiod washout to maintain consistent physiologic and metabolic conditions across periods, as expected in crossover bioavailability studies [1,2].

Screening, testing, and ongoing compliance monitoring

- Pre-admission affirmation and direct observation: Participants will be reminded of prohibitions and will attest to compliance at each check-in; during confinement, staff will directly observe permitted intake and behaviors per unit SOPs [1].
- Alcohol testing: Breath alcohol testing at screening and each admission; any positive result leads to cancellation of dosing for that period and evaluability review [1,2].
- Urine drug screening: A validated multi-analyte panel at screening and each admission will test for cannabinoids, cocaine metabolites, amphetamines/methamphetamines/MDMA, opiates/oxycodone/methadone, benzodiazepines, barbiturates, and phencyclidine at federally recognized cutoffs; confirmatory testing will follow reactive screens as per laboratory SOPs aligned to HHS workplace drug testing standards [5].
- Nicotine exposure testing: Urine or saliva cotinine testing at screening and each admission; results must be negative according to assay-specific thresholds to confirm abstinence from nicotine/tobacco products, recognizing cotinine's biomarker utility and multi-day detectability following exposure [6,14].
- Source documentation: All tests, participant attestations, and staff observations will be recorded contemporaneously in source documents and monitored for protocol compliance per ICH-GCP [3].

Handling of positive findings and noncompliance

- Alcohol positive, drug screen positive, or cotinine positive at screening: The participant will be screen-failed unless the investigator documents a clearly benign, non-prohibited explanation with negative confirmatory testing (e.g., legitimate prescribed medication inadvertently taken before consent), which is generally incompatible with the healthy volunteer restrictions for this study [1–3,5].
- Positive tests at admission or in-clinic use: Dosing for that period will be withheld; the participant may be discontinued for noncompliance. If safety allows and windows permit, a single rescheduling of a period may be considered after documented return to negative status and completion of the full protocol-specified washout; such decisions will be documented prior to unblinded PK analysis, consistent with bioavailability guidance and GCP [1–3].
- Protocol deviations: All deviations will be documented with assessment of impact on PK interpretability; periods affected by prohibited substance exposure may be excluded from per-protocol PK analyses, with handling prespecified in the statistical analysis plan [1,2].

Rationale and scientific considerations

- Caffeine/xanthines can acutely increase blood pressure and heart rate, adding variability to cardiovascular safety endpoints collected in proximity to Cmax [10].
- Tobacco/nicotine products and e-cigarettes alter salivary flow and oral mucosal physiology, which may affect the extent and rate of sublingual absorption; standardization is critical for transmucosal studies [4,14].
- Grapefruit/Seville orange products and St. John's wort are prototypical modulators of intestinal/hepatic CYP3A and P-gp with effects persisting beyond discontinuation; avoidance reduces inter- and intra-subject variability in crossover PK comparisons [2,7,8].

- Cannabinoids (including CBD) can inhibit multiple CYP enzymes and transporters, with potential to alter disposition of coadministered drugs and confound PK assessments; abstinence and negative testing minimize this risk [2,9].
- The specified washouts and testing framework reflect interpretive synthesis of bioavailability/bioequivalence guidance, DDI principles, and oral transmucosal pharmacology to preserve internal validity in this healthy volunteer crossover design [1,2,4,11].

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5.3.3 Physical Activity

Activity restrictions preadmission and during confinement

- To minimize within-subject variability in absorption, distribution, and cardiovascular responses, participants must refrain from strenuous or endurance-type exercise (e.g., running, high-intensity interval training, heavy resistance training) for at least 24 hours before

each dosing and until completion of the last pharmacokinetic (PK) sample in that period; routine light activities of daily living are permitted before admission [1,2]. Exercise can alter splanchnic blood flow and gastric emptying and thereby affect oral/sublingual drug absorption, and can modify drug disposition through changes in plasma volume and tissue perfusion [4,6].

- During each in-clinic confinement on dosing days, participants will remain under continuous supervision from at least 10 hours predose through completion of the 8-hour PK sampling window. Only quiet, low-exertion activities (reading, screen use without exertion) are permitted. No stretching sessions, calisthenics, yoga, or fitness activities are allowed [1,2].

Posture, ambulation, and confinement on dosing days

- From 1 hour predose until 2 hours postdose, participants will remain seated upright or semi-supine except for restroom visits; talking will be minimized during the first 15 minutes postdose to avoid perturbing sublingual residence of the drops. Thereafter, seated or semi-supine rest is required until 4 hours postdose; brief, slow ambulation to the restroom with staff escort is permitted as needed. From 4 to 8 hours postdose, light ambulation within the unit is allowed but no brisk walking or stair climbing [2,5].
- To reduce postural effects on plasma volume and PK parameters, the same posture (seated or semi-supine) should be maintained for at least 10 minutes before each scheduled blood draw and remain consistent across timepoints within a period; deviations will be recorded with exact timing [2].
- For the intravenous period, participants will keep the dosed arm supported and relatively still for 30 minutes postdose to protect cannula integrity; overall posture and ambulation requirements are as above [1,2].

Rest and sleep schedule around PK sampling

- Napping is not permitted from 1 hour predose until completion of the 2-hour postdose assessments to facilitate safety monitoring near anticipated tmax. Quiet rest with eyes open is allowed. After 2 hours postdose, brief naps are allowed if they do not interfere with scheduled procedures. Overnight lights-out will follow unit standard and will not conflict with PK sampling [1,2].

Rationale for activity standardization

- Acute exercise reduces splanchnic blood flow and can slow or variably affect gastrointestinal transit and oral drug absorption; standardization of activity and posture is recommended in bioavailability/bioequivalence trials to limit physiologic variability [2,4,6]. Sublingual delivery is sensitive to salivary flow and mucosal contact; dehydration and exertion-related changes in saliva can influence transmucosal uptake, supporting early postdose rest and minimized talking/swallowing [5]. This subsection reflects protocol-level interpretive synthesis integrating regulatory guidance with exercise pharmacokinetic literature to preserve internal validity [2,4–6].

Compliance documentation and handling of deviations

- Compliance with activity restrictions will be ensured by direct observation and recorded in nursing flowsheets and activity logs, including posture at each PK draw, times of any ambulation, and any unscheduled exertion. Predose affirmation of 24-hour exercise

abstinence will be obtained at admission. Source documentation will follow ICH-GCP requirements for contemporaneous, attributable records [3].

- Any deviation (e.g., unscheduled vigorous activity, prolonged upright ambulation within the first 2–4 hours, posture changes within 10 minutes before sampling) will be time-stamped in source documents and the electronic case report form, with an investigator assessment of potential impact on PK interpretability. Consistent with bioavailability guidance, periods materially affected by noncompliance may be excluded from per-protocol PK analyses per the statistical analysis plan, with decisions documented prior to unblinded analysis [1,2,3].

References

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5.3.4 Contraception and Pregnancy Testing

Eligibility status and definitions

- Women who are pregnant or breastfeeding are not eligible. Women of childbearing potential (WOCBP) must have a negative pregnancy test at screening and prior to each dosing period and must comply with highly effective contraception requirements detailed below [1–3].
- WOCBP is defined as sexually mature females who have not undergone documented hysterectomy or bilateral oophorectomy and who have not been postmenopausal for at least 12 consecutive months without an alternative medical cause; women on hormone replacement therapy with withdrawal bleeding are considered of childbearing potential [3].
- Given limited human pregnancy data for atropine and the ethical obligation to minimize potential fetal exposure in nontherapeutic healthy-volunteer research, pregnancy and lactation are exclusionary, and effective contraception is required [1,2,4].

Pregnancy testing procedures

- Assay type and sensitivity: Urine or serum human chorionic gonadotropin (hCG) testing using a validated assay with a detection limit approximately 20–25 mIU/mL for urine or per central laboratory specifications for serum will be used, per site standard operating procedures (SOPs) [2].
- Schedule:

- Screening: One pregnancy test within 28 days before first dosing (Day 1) [2].
- Predose testing: A pregnancy test will be performed within 24 hours prior to dosing in each study period (Days 1, 8, and 15) with results available and negative before administration of study treatment [2].

- Additional testing: If a dosing visit is delayed by >24 hours after a negative test, if pregnancy is suspected at any time, or if there is a protocol deviation increasing pregnancy risk, repeat testing will be performed before dosing [2].

- Documentation: Results will be recorded in source documents and the electronic case report form (eCRF) in accordance with ICH-GCP [1].

Contraception obligations for WOCBP

- Required effectiveness level: WOCBP must use a highly effective contraceptive method resulting in a failure rate of <1% per year when used consistently and correctly, or must practice true sexual abstinence that is the participant's usual and preferred lifestyle; periodic abstinence (calendar, ovulation tracking), withdrawal, and spermicide-only methods are not acceptable [2,6].
- Acceptable highly effective methods include the following, used singly unless otherwise noted [2,6]:

- Intrauterine device (IUD) (copper or levonorgestrel-releasing).
- Hormonal implant (etonogestrel).
- Bilateral tubal occlusion/ligation (documented).
- Established hormonal contraception with demonstrated effectiveness window achieved per product labeling (combined estrogen-progestin oral, transdermal, or vaginal ring; progestin-only pill; depot medroxyprogesterone acetate).
- Vasectomized partner with documented azoospermia as the sole partner, plus an additional barrier method for 90 days post-vasectomy if within the window of potential residual fertility [2,6].
- Sexual abstinence as a consistent lifestyle choice [2].

- Initiation and timing:

- Long-acting reversible contraception (IUD, implant) must be placed and functional before screening or at least one full ovulatory cycle prior to first dose [2,6].
- Hormonal methods must be initiated and taken per label to achieve contraceptive effectiveness prior to first dose; if started or re-started within 30 days prior to first dose, a barrier method (male condom) must be added until the hormonal method's effectiveness window is achieved [2,6].

- Duration:

- Contraception must start before first dose as above and continue through at least 7 days after the last dose (covering substantially more than five terminal half-lives of atropine, which is approximately 2–4 hours after intravenous administration), after which no further study-mandated contraception is required beyond routine use [2–4].

- Oocyte/embryo donation: WOCBP must agree not to donate oocytes for assisted reproduction from consent through at least 7 days after last dose [2,3].

Contraception and donation obligations for male participants

- Male participants who are sexually active with a partner of childbearing potential must either remain abstinent as a consistent lifestyle choice or use a male condom during intercourse from first dose until 7 days after last dose; an additional highly effective method used by the female partner is recommended to further reduce the risk of pregnancy [2,3].
- Men must refrain from sperm donation from consent until 7 days after last dose [2,3].
- These timeframes are based on the short elimination half-life of atropine and the absence of evidence for germ cell toxicity; requirements are conservative to minimize any theoretical risk of drug exposure to an embryo/fetus via seminal fluid or early conception [2–4]. This reflects protocol-level interpretive judgment aligning contraception duration with pharmacokinetics and international guidance.

Procedures for positive pregnancy test or pregnancy during the study

- Positive test at screening or predose: The participant will be screen-failed or not dosed, respectively, and will be withdrawn from further participation [2].
- Pregnancy discovered after dosing:

- Immediate actions: Study treatment will not be administered in any subsequent period. The investigator will inform the participant, ensure appropriate counseling and referral to obstetric/gynecologic care, and discuss potential risks and available information from the atropine labeling [1,4].
- Follow-up: The pregnancy will be followed to outcome with participant consent, including neonatal outcome, and any complications will be documented. Pregnancy itself is not considered an adverse event; however, any associated maternal, fetal, or neonatal complication meeting seriousness criteria will be reported in accordance with applicable safety reporting requirements and ICH-GCP [1,5].
- Regulatory/ethics notifications: Reporting to the sponsor, institutional review board/independent ethics committee (IRB/IEC), and competent authorities will follow local requirements and FDA guidance for IND safety reporting, as applicable to bioavailability/bioequivalence studies [1,5].

Compliance assessment

- At each visit, staff will verify ongoing adherence to contraception or abstinence requirements and document any changes in sexual activity or contraceptive use. Noncompliance will trigger risk assessment, counseling, pregnancy testing as indicated, and potential discontinuation from the study to protect participant safety and data integrity [1,2].

References

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5.4 Screen Failures

Definition and scope

- A screen failure is any consented individual who undergoes one or more protocol-specified screening procedures but is not enrolled/randomized because at least one inclusion criterion is not met, at least one exclusion criterion is met, a required assessment is missing or indeterminate, a prohibited condition or product use is identified, or the individual withdraws from screening prior to enrollment [1,2].

Documentation requirements

- The site will maintain a contemporaneous screening and enrollment log capturing, at minimum: unique subject identification code, date of informed consent, screening window dates, enrollment/randomization status, and the primary reason category for screen failure (e.g., medical history/diagnosis, physical exam/vitals, ECG, laboratory assessments, pregnancy test, substance testing, prohibited medications/substances, noncompliance with lifestyle restrictions, withdrawal of consent, other administrative) [1,5].
- For each screened individual, source documents will include signed informed consent, eligibility checklist, medical history and concomitant medication review, physical examination and vital signs, 12-lead ECG report, clinical laboratory and pregnancy test results, substance testing (alcohol, drugs of abuse, cotinine) results, and investigator eligibility determination. The subject identification code list linking the coded identifier to the subject's personal identity will be maintained separately and securely to protect confidentiality, per ICH-GCP [1].
- Electronic source and eCRF entries must be attributable, legible, contemporaneous, original, and accurate, with audit trails enabled; validation and user access controls will follow FDA recommendations for electronic source data in clinical investigations and site SOPs [1,6].

Data handling and analysis set considerations

- Screen-failure data will not be included in efficacy or pharmacokinetic analyses and will be summarized only in disposition reporting (e.g., numbers screened, excluded, and enrolled; primary reasons for exclusion) to support transparency in line with CONSORT guidance for participant flow. This paragraph reflects protocol-level interpretive practice to align reporting with established trial reporting guidelines [5].

Record storage and retention

- The investigator will retain all screen-failure records, including screening logs, eligibility checklists, consent forms, and related source documents, in accordance with ICH-GCP essential document retention requirements and applicable U.S. regulations. At minimum, records will be retained until the later of: (a) 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications, or (b) 2 years after formal discontinuation of clinical development and notification to regulators, as applicable [1,3]. For bioavailability/bioequivalence investigations, maintenance of records pertinent to in vivo testing must additionally comply with 21 CFR 320.36 [4].
- Records will be stored in secure, access-controlled archives (paper and/or validated electronic systems) with environmental and disaster recovery protections per site SOPs; access will be limited to authorized personnel, the sponsor and its designees, institutional review board/independent ethics committee (IRB/IEC), and regulatory authorities for monitoring, audit, and inspection [1,6].

Conditions for rescreening

- Rescreening may be permitted at the investigator's discretion for otherwise eligible individuals whose initial screen failure is attributable to a transient or correctable condition (e.g., isolated laboratory value marginally out of range, temporary intercurrent illness, incomplete documentation) and when study timelines and screening windows allow. Rescreening is limited to one additional attempt unless specifically approved by the sponsor in writing; all prior screening results will remain part of the permanent record [1].

- Minimum intervals and prerequisites for rescreening are as follows, aligned to protocol restrictions and to preserve data integrity:

- Positive alcohol test: at least 24 hours since last alcohol intake with a documented negative breath alcohol test and affirmation of compliance before any repeat procedures [2].
- Positive drug screen or cotinine screen: documented negative confirmatory test and completion of the full protocol-specified washout for the substance class (e.g., cannabinoids and nicotine/tobacco products require at least 30 days of abstinence before rescreening per study restrictions), with investigator assessment that ongoing use is unlikely [2].
- Prohibited prescription/OTC/herbal products: completion of the full protocol-specified washout (e.g., 28 days for strong/moderate enzyme/transport inducers; 14 days for strong/moderate inhibitors; 30 days for anticholinergic/cholinomimetic agents) and verification of discontinuation, consistent with study restrictions and DDI guidance [2].
- Vital signs or ECG out of range: documented return to acceptable ranges and absence of disqualifying cardiovascular findings on repeat assessment; any clinically significant abnormality remains exclusionary [2].
- Pregnancy test positive or indeterminate: pregnancy is exclusionary; rescreening is not allowed within the same screening window. If a future participation is considered, it requires a new consent and eligibility assessment after resolution (negative test) and adherence to contraception requirements [1,2].

- All rescreening approvals and timing will be documented in source with rationale prior to conducting repeat procedures. The same consent requirements apply to rescreening activities, and any repeated assessments will follow the same methods and laboratories to ensure comparability [1].

Regulatory reporting and audit readiness

- The screening log and screen-failure source records will be made available for sponsor monitoring and for inspection by regulatory authorities and IRB/IEC, supporting verification that enrollment adhered to the approved inclusion/exclusion criteria and that no investigational product was administered to ineligible individuals [1,3,4].
- Numbers screened, excluded, and enrolled, and primary reasons for exclusion will be captured in the eCRF and summarized in study reports, consistent with FDA bioavailability guidance expectations for transparent study conduct description and with CONSORT-style participant flow reporting [2,5].

Operational transition to intervention

- Only subjects who meet all eligibility criteria at the end of screening and at the pre-dose confirmation check will proceed to randomization and first dosing. Any subject with unresolved eligibility uncertainties at admission will not be dosed and will be managed as a screen failure or rescreen per the above procedures, ensuring that only eligible participants receive sublingual or intravenous atropine in the subsequent study intervention phase [1,2].

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6 STUDY INTERVENTION(S) AND CONCOMITANT THERAPY

6.1 Study Intervention(s) Administered

Overview of study interventions and design

- This randomized, open-label, single-dose, three-sequence, three-period crossover study administers two sublingual doses of atropine sulfate ophthalmic solution USP 1% (test product; investigational use) and one intravenous dose of atropine sulfate injection (reference product) under standardized conditions to compare the rate and extent of systemic exposure across routes and doses in healthy adults [1,2].
- Each subject receives all three treatments across Periods 1–3, separated by approximately 7-day washouts, randomized 1:1:1 to sequences A (low sublingual → high sublingual → IV), B (high sublingual → IV → low sublingual), or C (IV → low sublingual → high sublingual) [1,2].
- All dosing is supervised by qualified study personnel, performed per site SOPs, and documented contemporaneously in source records consistent with ICH-GCP [3].

Test product: atropine sulfate ophthalmic solution USP 1% administered sublingually

- Pharmaceutical form and strength: Commercial atropine sulfate ophthalmic solution USP 1% (10 mg/mL) in multidose dropper bottles; sterility and preservative per labeling. This product is used investigationally for sublingual administration in this protocol [4].
- Dose levels and volumes:

- Low dose: 0.5 mg atropine base equivalent, administered as 0.05 mL (50 µL) of the 1% solution.
- High dose: 1.0 mg atropine base equivalent, administered as 0.10 mL (100 µL) of the 1% solution [4].

- Route and method of administration:

- Route: Sublingual (drops placed under the tongue).
- Delivery: A sterile, calibrated positive-displacement pipette or single-use metered micropipette will be used to withdraw and dispense the exact target volume from the commercial container to the sublingual space; the tongue will be gently elevated to allow direct placement. A second staff member will independently verify the volume setting and dose calculation prior to administration [1–3].
- Post-administration positioning and behavior: Subjects will remain seated upright or semi-supine for at least 2 hours postdose, minimizing talking and swallowing for the first 15 minutes to support consistent sublingual residence and absorption; restroom use is permitted with escort as needed [2].

- Number of dosage units and application area: A single administration per period consisting of the total period dose delivered as one continuous sublingual application targeting the floor of mouth under the tongue [2,4].
- Rationale and standardization: Sublingual administration of small volumes of solution enables rapid transmucosal uptake while bypassing first-pass metabolism; standardizing mucosal contact and early postdose behavior minimizes variability in Tmax and Cmax for bioavailability comparisons [2,6].

Reference product: atropine sulfate injection 0.4 mg/mL administered intravenously

- Pharmaceutical form and strength: Commercial atropine sulfate injection 0.4 mg/mL in sterile solution for intravenous use [5].
- Dose and volume: 1.0 mg atropine base equivalent administered as 2.5 mL of the 0.4 mg/mL solution [5].
- Route and method of administration:

- Route: Intravenous (IV).
- Delivery: Single IV bolus via an indwelling peripheral venous cannula placed prior to dosing. The cannula will be flushed per site SOPs to confirm patency; the bolus will be administered by qualified staff with continuous observation and immediate availability of resuscitation equipment, consistent with product labeling and early-phase unit standards [3,5].

- Number of dosage units: A single IV bolus dose per period [5].

Dosing conditions and standardization across periods

- Fasting and fluids:

- No food or drink from 1 hour before dosing until 1 hour after dosing; the first standardized meal will occur no earlier than 4 hours postdose. Limited sips of water are permitted for safety assessments or swallowing concomitant rescue medications if medically indicated, with timing and volume recorded [1,2].

- Posture and activity:

- From 1 hour predose through at least 2 hours postdose, subjects remain seated upright or semi-supine except brief restroom visits; the same posture is maintained for at least 10 minutes prior to each scheduled PK draw within a period. Light ambulation may be permitted after 2 hours postdose without exertion, with timing documented to preserve PK interpretability [2].

- Concomitant intake and restrictions:

- No alcohol within 24 hours before dosing until collection of the final PK sample for the period; no caffeine/xanthines from 24 hours before dosing through the last PK sample in the period; prohibited medications and substances per protocol remain disallowed throughout. These restrictions are intended to minimize physiologic and metabolic variability for crossover comparisons [1,2].

- Supervision and safety monitoring:

- Continuous on-unit supervision from at least 10 hours predose through completion of the 8-hour PK sampling window; serial vital sign and ECG monitoring will be performed per schedule, with readiness for clinical management of anticholinergic effects as per product labeling and unit SOPs [1,3,5].

Procedures to ensure accurate dosing, full dose delivery, and compliance

- Dose preparation and verification:

- Sublingual doses: The commercial ophthalmic container will be gently inverted per labeling to ensure homogeneity. The exact volume (50 µL or 100 µL) will be measured using a calibrated, certified device appropriate for micro-volumes; calibration records will be current. Two-person independent verification of subject identity, treatment, sequence/period, dose, and volume will occur before administration, with documentation in source and drug accountability logs [1–3].
- Intravenous dose: The required 2.5 mL will be drawn into a sterile syringe from the commercial vial/ampule using aseptic technique, labeled with subject ID, treatment, dose, and time. Two-person verification will confirm identity, treatment, and volume prior to administration [1–3,5].

- Administration control and confirmation:

- Sublingual: Staff will visually confirm full deposition of the solution under the tongue and absence of immediate expectoration; any coughing, swallowing, or drooling within the first 2 minutes postdose will be recorded with estimated loss, and the period may be assessed for evaluability in the SAP. No water rinse is permitted within 1 hour postdose unless medically necessary; all exceptions are documented [1,2].
- Intravenous: Patency will be confirmed by gentle aspiration or saline flush before and after the bolus. Start and stop times, any infiltration, and line issues will be documented; any deviation will be assessed for impact on PK [1–3,5].

- Time anchoring and documentation:

- The exact clock time of dose administration (end of sublingual deposition; start and stop of IV bolus) will be the reference for PK sampling. All predose checks (fasting status, pregnancy testing for WOCBP, alcohol/drug/cotinine testing as applicable), posture, and restrictions will be confirmed and recorded prior to dosing [1–3].

- Accountability and chain of custody:

- Receipt, storage (temperature, light), preparation, dispensing, and reconciliation of all commercial products will be recorded per ICH-GCP and site SOPs; any wastage or spillage will be quantified and documented. Only unopened, in-date containers/lots with verified labeling will be used for dosing [1,3].

- Compliance oversight:

- Dosing will occur only after eligibility reconfirmation and verification of protocol-specified restrictions. Direct observation will ensure adherence to immediate postdose behavior (e.g., minimal talking/swallowing for the first 15 minutes after sublingual dosing). Deviations will be time-stamped in source, with investigator assessment of potential impact on PK and per-protocol evaluability defined in the SAP [1–3].

Alignment with scientific and regulatory expectations

- The above administration procedures and restrictions are designed to reduce intra- and inter-subject variability, enabling interpretable comparisons of AUC and Cmax between sublingual and intravenous routes in a crossover design, consistent with FDA bioavailability and EMA bioequivalence guidance for standardized dosing conditions, fasting status, posture control, and rigorous documentation in early-phase PK studies [1,2]. The sublingual technique and early postdose minimization of swallowing/talking reflect interpretive synthesis of mucosal delivery principles to enhance consistency of transmucosal uptake in a controlled clinical pharmacology setting [2,6].

References

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6.1.1 Administration and Dosing Schedule

Dosing regimen, sequences, and timing

- Randomized, open-label, single-dose, three-period crossover with 1:1:1 allocation to sequences A (low sublingual → high sublingual → IV), B (high sublingual → IV → low

sublingual), or C (IV → low sublingual → high sublingual). Dosing occurs on Days 1, 8, and 15 with approximately 7-day washouts between periods [1,2].

- Treatments per period:

- Low-dose sublingual: atropine sulfate ophthalmic solution USP 1% (10 mg/mL), 0.5 mg (50 µL) placed under the tongue once.
- High-dose sublingual: atropine sulfate ophthalmic solution USP 1% (10 mg/mL), 1.0 mg (100 µL) placed under the tongue once.
- Intravenous: atropine sulfate injection 0.4 mg/mL, 1.0 mg (2.5 mL) as a single IV bolus via an indwelling peripheral venous cannula [3,4].

Pre-dose restrictions, admission, and hydration

- Admission and supervision: Participants are admitted to the phase 1 unit no later than 10 hours before each dosing and remain under continuous supervision until completion of the 8-hour PK sampling window for that period, consistent with early-phase PK standardization to limit physiologic variability [1,2].
- Fasting and fluids: No food or drink (including water) from 1 hour before dosing until 1 hour after dosing; the first standardized meal occurs no earlier than 4 hours postdose. Water is permitted ad libitum until 1 hour predose to ensure euhydration; no water is permitted during and for 1 hour after sublingual dosing to standardize mucosal exposure. Limited sips of water for essential procedures may be allowed with exact timing and volume documented [1,2].
- Alcohol, caffeine, nicotine, and medications: No alcohol within 24 hours before dosing until collection of the last PK sample for the period; no caffeine/xanthines and no nicotine/tobacco per protocol lifestyle restrictions; no prescription/OTC/herbal products as specified in exclusion criteria and washouts [1,2].
- Pre-dose checks: Prior to each dosing, confirm continued eligibility, vital signs within acceptable ranges, negative pregnancy test for WOCBP within 24 hours, compliance with restrictions, patency of IV cannula when applicable, and completion of all predose PK and safety assessments. Confirmation and time-stamped documentation are required per ICH-GCP [5].

Dose administration procedures and definition of time zero

- Identity and dose verification: A two-person check will confirm subject identity, assigned sequence/period treatment, dose, and volume prior to administration; verification is documented in source and accountability records in real time with audit trails [5,6].
- Sublingual administration (test product):

- Using a sterile, calibrated positive-displacement pipette/metered micropipette, dispense the exact volume (50 µL or 100 µL) under the elevated tongue onto the floor of the mouth in a single, continuous application. The subject minimizes swallowing and talking for 15 minutes postdose to standardize early mucosal residence, per transmucosal delivery best practices [2,3].
- Time zero (t0) is defined as the exact clock time at completion of sublingual deposition. Start of deposition and t0 are both recorded to the second when possible; if to the minute, record seconds as ":00" and state resolution in source [1,2,6].

- Intravenous administration (reference product):

- Administer 1.0 mg (2.5 mL) as a single IV bolus via an indwelling peripheral venous cannula; confirm patency and flush per SOP. Record start and stop times of the bolus. Time zero (t0) is defined as the exact clock time at completion of the IV bolus [3,5].

- Synchronization and documentation: All dosing and sampling times use a single synchronized time source (site atomic-synced clock). Start/stop of dosing, and any dosing-related events (coughing, drooling, suspected loss, infiltration) are contemporaneously recorded and verified by a second staff member, consistent with FDA electronic source data guidance and ICH-GCP ALCOA+ principles [5,6].

Post-dose posture and observation

- From 0 to 2 hours postdose, subjects remain seated upright or semi-supine except brief restroom visits; minimize talking/swallowing during the first 15 minutes after sublingual dosing. From 2 to 4 hours, seated/semi-supine rest continues with brief escorted ambulation as needed. From 4 to 8 hours, light ambulation within the unit is permitted. Maintain the same posture for at least 10 minutes before each PK blood draw to reduce postural effects on PK parameters. Observation continues through the 8-hour PK window, with vital signs and heart rate/blood pressure monitoring for at least 4 hours postdose [1,2].

Dosing day schedule and PK sampling

- The PK sampling schedule is anchored to t0 for each period. Target nominal times: predose (within 60 minutes before t0) and at 2, 4, 6, 10, 15, 20, 30, 45, 60 minutes, and 2, 4, 6, and 8 hours postdose [1,2].
- Operational windows:

- Early samples (≤60 minutes): aim for exact nominal time; acceptable deviation is ±2 minutes. Record actual time for concentration–time analysis relative to t0 [1,2].
- Later samples (>60 minutes): acceptable deviation is ±5 minutes. Larger deviations are documented as protocol deviations with impact assessment on PK evaluability (see below) [1,2,5].

1. Sample time recording and verification: For each draw, record the actual clock time of venipuncture or vacutainer fill start as the sample collection time; if pre-collected via an indwelling line, record line flush time and sample collection start/stop. All times are double-checked against the synchronized time source and entered into eSource/eCRF with automatic audit trails per FDA guidance [5,6].

Study Day Activity	Relative Time to t0	Target Clock Time Example	Notes
Admit to unit, eligibility reconfirmation, predose assessments	t0 −10 h to −2 h	Evening prior or morning	Includes pregnancy test (WOCBP), vitals, ECG as applicable
Begin fasting (no food/drink)	t0 −1 h	07:00 if t0 at 08:00	Water allowed until this time
Predose PK sample	t0 −0.5 to −0.0 h	07:30–08:00	Record exact time
Dose administration (define t0)	t0	08:00	SL: end of deposition; IV: end of bolus
PK samples	2, 4, 6, 10, 15, 20, 30, 45, 60 min; 2, 4, 6, 8 h	08:02 … 16:00	Record actual time for each draw
Resume water	t0 +1 h	09:00	Standardized meal ≥t0 +4 h
Standardized meal	t0 +4 h or later	≥12:00	Per site menu
End of period observation	t0 +8 h	16:00	Discharge if clinically appropriate

- Sample handling: Process, storage, and shipment follow site SOPs with time from collection to processing recorded; any delays are documented and assessed for potential impact per GCP [5].

Washout interval and justification

- A washout of approximately 7 days separates each single-dose period. Atropine exhibits a terminal half-life of about 2–4 hours after IV administration; a 7-day interval corresponds to >40–80 half-lives, which exceeds the ≥5 half-life standard recommended by EMA and FDA to

preclude carryover of parent drug or pharmacodynamic effects in crossover designs [1–4]. The 7-day washout also accommodates full reversal of anticholinergic effects and operational scheduling.

Management and documentation of dosing deviations

- General principles: All deviations are documented in source and eCRF with exact timing, description, root cause (if known), and immediate actions. The investigator assesses potential impact on PK interpretability prior to database lock, consistent with FDA BA guidance and ICH-GCP [1,5].
- Delayed or mistimed dosing:

- If dosing cannot occur at the planned clock time, proceed when ready and re-anchor all PK times to the actual t0. If predose conditions (fasting window, pregnancy test validity) lapse, re-establish requirements before dosing. If dosing is deferred to another day, ensure the protocol-specified washout is preserved between adjacent periods; update the randomization schedule accordingly and document [1,2,5].

- Incomplete dosing or dosing loss (sublingual):

- Record any coughing, expectoration, drooling, or swallowing within the first 2 minutes postdose, with an estimate of potential loss (none, minimal, moderate, substantial) and observed residues. Do not re-dose within a period. Such periods are typically excluded from per-protocol PK analyses due to uncertain delivered dose but retained for safety and may be included in sensitivity analyses per SAP [1,2].

- IV infiltration or extravasation:

- Document start/stop times, estimated delivered volume, signs of infiltration, and any intervention. If incomplete delivery is suspected, exclude the period from per-protocol PK; include in safety set. Do not administer a replacement dose in the same period [1,2,5].

- Emesis/vomiting:

- For orally/sublingually administered immediate-release products, if vomiting occurs at or before 2 times the median tmax for the corresponding treatment, exclude the affected period from per-protocol PK evaluation; if after this time, include unless other factors compromise data integrity. Record the exact time of emesis and characterize contents if feasible (e.g., presence of fluid consistent with study drug) [2]. This approach follows EMA bioequivalence guidance and is adopted here for bioavailability interpretability.

- Missed or mistimed PK samples:

- Collect at the earliest feasible time and record the actual time. Isolated missing samples generally do not preclude PK evaluation if overall coverage is adequate to characterize Cmax and the terminal phase; multiple missed early samples may preclude reliable Cmax/tmax estimation and lead to exclusion from per-protocol PK for that period. Impact is predefined in the SAP consistent with FDA BA guidance [1].

- Protocol noncompliance affecting absorption or disposition:

- Deviations from fasting, prohibited substances, posture requirements proximal to sampling, or significant physical exertion are documented with timing. If likely to materially affect PK (especially within the absorption window), the period may be excluded from per-protocol PK and included in sensitivity analyses; all decisions are documented prior to unblinding of sequence effects [1,2,5].

Analysis set assignment linked to dosing conduct

- Safety set: All participants who receive any study treatment are included in safety analyses according to treatment received [1,5].
- PK full analysis set (PK-FAS): All dosed periods with sufficient concentration–time data and without critical dosing errors; actual sampling times are used in noncompartmental analyses [1,2].
- PK per-protocol set (PK-PPS): Subset of PK-FAS excluding periods with major deviations impacting dose delivery or PK interpretability (e.g., emesis ≤2× median tmax for sublingual dosing; incomplete dosing; IV infiltration with uncertain full delivery; substantial timing deviations for early PK; noncompliance with fasting/behavioral restrictions within the absorption window), consistent with regulatory expectations for bioavailability assessments

[1,2]. Assignment is finalized blinded to treatment comparisons and documented in the data review plan/SAP.

References

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6.2 Preparation, Handling, Storage, and Accountability

Roles and responsibilities

- The principal investigator delegates day-to-day investigational product (IP) management to the study pharmacist or qualified designee, who oversees receipt, verification, storage, preparation, dispensing, reconciliation, and disposition. All activities are documented contemporaneously and are available for monitoring and inspection per ICH-GCP and U.S. regulations [1,3,6].

Product receipt and verification

2. Shipments are received by designated pharmacy staff and checked against the packing list for product identity (atropine solfate ophthalmic solution USP 1% [NDA 206289]; atropine solfate injection 0.4 mg/mL [NDA 209260]), strength, dosage form, quantity, manufacturer, lot/batch number, and expiry date. Any evidence of damage, compromised container closure, or labeling discrepancies leads to immediate quarantine and notification to the sponsor.
3. Temperature exposure during transit is reviewed from shipping indicators/data loggers where provided. If an excursion outside labeled storage conditions is suspected, the shipment is quarantined pending sponsor/manufacturer guidance, consistent with good storage and distribution practices for time- and temperature-sensitive pharmaceuticals [7].
4. Upon acceptance, products are entered into the site's IP inventory log with date/time received, condition on receipt, lot/expiry, and initial balance, signed/dated by the receiving staff member [1,6].

Labeling, period identification, and chain of custody

- Although this study is open-label, each dispensed unit (syringe for IV or prepared dosing set for sublingual) is labeled with, at minimum: protocol identifier, subject ID, sequence/period, treatment (test/reference), route, dose/volume, lot number, expiry, storage conditions, preparation date/time (if applicable), and “For investigational use only.” Labeling practices conform to GCP and, where applicable, 21 CFR 312.6 [1,4].
- A two-person verification documents subject identity, assigned sequence/period, product, dose/volume, and lot/expiry at the time of dispensing and immediately prior to administration. Chain of custody from pharmacy to dosing area and back (for returns/waste) is logged with date/time, quantities issued/returned, and signatures [1,6].

Storage conditions and environmental monitoring

- General controls:

- Store in secure, access-controlled areas with continuous temperature monitoring and calibrated devices; maintain records and investigate excursions. Products remain in original manufacturer packaging to preserve labeling and light protection until preparation/dispensing [1,7].
- Segregate quarantined, expired, damaged, or study-use-only supplies from usable stock with clear labeling and restricted access [1].

- Atropine sulfate ophthalmic solution USP 1% (test product):

- Manufacturer-specified storage: Store at controlled room temperature 20–25°C (68–77°F); excursions permitted to 15–30°C (59–86°F). Protect from light. Keep bottle tightly closed. Do not freeze. Avoid contaminating the dropper tip [1].
- Site application: Maintain in light-protective secondary containers when outside original cartons; limit time at ambient light during preparation/dispensing.

- Atropine sulfate injection 0.4 mg/mL (reference product):

- Manufacturer-specified storage: Store at 20–25°C (68–77°F); excursions 15–30°C permitted. Protect from light; keep in carton until use. Do not freeze. Single-dose presentation—use promptly after opening and discard any unused portion [2].
- Site application: Retain vials/ampules in cartons until preparation; use amber bags or light-protective covers for labeled syringes if transient delays before dosing are anticipated.

Preparation and handling procedures

- Aseptic technique and PPE:

- All IV dose preparations are performed using aseptic technique by qualified personnel wearing appropriate PPE (gloves, protective eyewear if splash risk). While atropine is not listed by NIOSH as a hazardous drug, standard precautions are used to prevent accidental exposure and cross-contamination [8].

- Test product (sublingual administration of ophthalmic solution 1%):

- Prior to withdrawal, gently invert the bottle per labeling to ensure homogeneity; do not shake vigorously [1].
- Using a sterile, calibrated positive-displacement pipette or single-use metered micropipette with a sterile tip, withdraw exactly 0.05 mL (50 µL) for the 0.5 mg dose or 0.10 mL (100 µL) for the 1.0 mg dose into a sterile transfer device immediately before administration. Do not allow the bottle tip or pipette tip to contact any non-sterile surface; discard tips after single use [1].
- Prepare doses individually at point of care; do not pre-aliquot for later use. If an aliquot is prepared minutes before dosing, protect from light and administer without delay; any prepared, unused aliquot is discarded and not returned to stock.

- Reference product (intravenous injection 0.4 mg/mL):

- Immediately prior to use, visually inspect the vial/ampule for particulate matter or discoloration; if present, do not use and quarantine the container [2].
- Using aseptic technique, withdraw 2.5 mL (1.0 mg) into a sterile syringe. Label the syringe with subject ID, dose, route, lot, and preparation time; keep protected from light until administration. As a single-dose presentation, any remaining solution in the opened container is discarded; do not pool residuals [2].

Dispensing and administration support

- Dispensing records document subject ID, sequence/period, product, lot/expiry, quantity dispensed, date/time dispensed, and preparer/verifier signatures. At administration, the nurse confirms identity, dose/volume, route, and timing against the source order and documents start/stop times, consistent with GCP [1,6].
- For the sublingual test product, the original commercial bottle remains pharmacy stock; only the measured aliquot and sterile delivery device are taken to the bedside. To prevent cross-contamination, no bottle is designated to an individual subject, and no bedside withdrawals from the manufacturer bottle are permitted [1].

Accountability, reconciliation, and recordkeeping

- The site maintains:

- IP inventory logs (receipt, lot/expiry, quantities on hand).
- Temperature monitoring logs and excursion investigations for all storage locations.
- Dispensing/return logs at the subject and period level.
- Preparation worksheets (dose calculation/volume, device calibration confirmation, preparer/verifier sign-off).
- Administration records (date/time, dose, route, product lot, observations).
- Wastage/destruction logs (quantity, lot, reason, method, date, authorized signatures).

- At predefined intervals and at study close-out, pharmacy staff reconcile quantities received, dispensed, returned, wasted, and remaining, generating a final reconciliation report signed by the investigator and made available to the sponsor/monitor. All records are retained per ICH-GCP and U.S. regulations for BA/BE investigations [1,6].

Management of temperature excursions, damaged, expired, or unused product

- Temperature excursions are promptly documented, products quarantined, and suitability for continued use determined in consultation with the sponsor/manufacturer, following good storage/distribution practices [7].
- Damaged containers, products with visible particulate/discoloration, expired stock, or products exposed to unacceptable conditions are segregated and not used for dosing. These are either returned to the sponsor or destroyed on site per institutional SOPs and regulatory requirements, with documentation and authorization [1,5,6].
- Unused prepared doses (e.g., opened ampules/vials or measured aliquots) are not returned to inventory and are discarded immediately in appropriate pharmaceutical waste streams, with quantities recorded [2].

Disposal

- Disposal follows institutional procedures and applicable regulations for pharmaceutical waste. For IND-regulated studies, disposition of IP (including destruction) occurs under the investigator’s responsibility with sponsor authorization and contemporaneous records, consistent with 21 CFR 312.59 and ICH-GCP [1,5]. Atropine is not a controlled substance; no DEA controls apply.

Measures to preserve study integrity and safety

- Although the study is open-label, segregation of test and reference products in storage and dispensing areas minimizes selection errors. Unique color-coded shelf labels and period-specific preparation worksheets are used to prevent mix-ups.
- Staff avoid self-exposure; any skin or mucosal contact is immediately washed with water. Spills are contained and cleaned using standard precautions; affected stock is assessed and quarantined if contamination is possible [8].
- Transport from pharmacy to dosing area uses closed, light-protected carriers with documented handoff to maintain chain of custody and environmental protection [7].

Documentation standards and retention

- All preparation, handling, storage, dispensing, reconciliation, and disposition records are contemporaneous, attributable, and auditable, maintained in source and pharmacy files. Records are retained in accordance with ICH-GCP and 21 CFR 312.62/320.36, and made available for sponsor monitoring and regulatory inspection [1,6].

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6.3 Measures to Minimize Bias: Randomization and Blinding

Overview and objectives

- This single-center, randomized, open-label, three-sequence, three-period crossover study uses prospective allocation concealment at enrollment and rigorous operational standardization across periods to minimize selection, performance, and measurement bias while enabling objective pharmacokinetic (PK) comparisons of sublingual versus intravenous atropine in healthy volunteers. Crossover randomization and balanced sequences are

required for valid within-subject bioavailability assessment and to mitigate period and carryover effects in line with regulatory expectations for BA/BE investigations [1,2].

Randomization scheme, method, and implementation

- Allocation unit and ratio:

- Participants are randomized 1:1:1 to one of three fixed treatment sequences (A: low SL → high SL → IV; B: high SL → IV → low SL; C: IV → low SL → high SL). Each subject remains on the assigned sequence for all three periods separated by ~7-day washouts [1,2].

- Method of sequence assignment:

- A centralized computer-generated randomization schedule using permuted blocks (undisclosed block size) stratified by sex (male/female) is produced to maintain near-equal allocation across sequences and reduce the risk of time-related imbalances in a single-center study [3,4]. The undisclosed block size and centralized assignment support allocation concealment at the point of enrollment [4].

- Schedule generation and validation:

- The schedule is generated by an independent, unblinded trial statistician at the sponsor or contracted CRO using validated statistical software with a documented random seed. Generation, quality control review, and sign-off are recorded in the trial master file (TMF) per ICH-GCP essential document standards [3].

- Allocation concealment and operationalization:

- Assignment is implemented via a secure interactive web response system (IWRS) or, if IWRS is unavailable, via sequentially numbered, tamper-evident, opaque envelopes prepared by the independent statistician. Site staff enroll eligible subjects and obtain the sequence assignment immediately prior to Period 1 dosing; the same assignment governs Periods 2–3. The allocation mechanism prevents foreknowledge of upcoming assignments and selection bias [3,4].

- Roles and access:

- Authorized unblinded roles: the independent statistician (schedule generation/maintenance), designated pharmacy staff (dispensing logistics), and a limited number of sponsor operations personnel responsible for IWRS administration. Investigators, clinical staff, monitors, and blinded statisticians responsible for PK analyses do not have access to treatment identities within the analysis data until database lock and finalization of the statistical analysis plan (SAP) sections subject to blind review, consistent with ICH guidance on bias minimization and blind data review practices [3].

Blinding approach and justification

- Open-label conduct:

- The study is open-label with respect to participants and site personnel because treatments differ by route and dose (sublingual micro-volume vs intravenous bolus), making blinding impractical. For BA studies with objective PK endpoints (AUC, Cmax, tmax, t½), open-label designs are acceptable; randomization and standardized procedures are the primary measures to control bias [1,2]. This approach aligns with FDA and EMA guidance for BA/BE investigations where pharmacokinetic measurements are not susceptible to observer expectation effects [1,2].

- Analytical blinding:

- To further reduce analysis bias, the PK bioanalytical laboratory quantifies atropine concentrations without knowledge of clinical treatment assignments, and the primary PK analysis is performed by statisticians blinded to treatment labels (e.g., masked as T1, T2, R) until data cleaning, derivation rules, and the SAP are finalized. This is an interpretive, protocol-specific practice grounded in principles from ICH E9 on minimizing analytic bias through pre-specification and, where feasible, blinding of analysts [3].

Maintenance, security, and changes to the randomization code

- Schedule custody and version control:

- The master randomization list and audit trail (including seed, software version, generation outputs, QC sign-off, and any IWRS configuration) are maintained under document control by the independent statistician or IWRS vendor with role-based access. Any amendments (e.g., extension blocks to accommodate replacement enrollments) require predefined approval workflows and are versioned, with superseded versions retained in the TMF [3].

- Site-level records:

- The site maintains a subject identification code list and enrollment/randomization log distinct from source data, stored in secure, access-controlled files. Records are retained per ICH-GCP and U.S. regulations for BA/BE studies (21 CFR 312.62; 21 CFR 320.36) and made available for monitoring and inspection [3,5,6].

- Protection from unauthorized access:

- All randomization and allocation documentation (electronic and paper) is stored in access-restricted repositories with audit trails, periodic backups, and disaster recovery controls. IWRS access is limited to trained, authorized users with unique credentials; permissions are configured to prevent blinded roles from seeing treatment identities [3].

Emergency unblinding

- Given the open-label design, there is no treatment blind to break at the site level. If masked labels are used for analysts, emergency unmasking of analysis labels is not anticipated and would not affect clinical care. Any inadvertent disclosure of masked labels prior to SAP finalization is documented, assessed for impact, and reported to the sponsor; corrective and preventive actions are implemented to preserve the integrity of the analysis set, consistent with ICH-GCP documentation principles [3].

Additional measures to minimize bias

- Period effects and carryover:

- Randomized sequence allocation and adequate washouts (>5 half-lives) mitigate systematic period and carryover effects as recommended in BA/BE guidance; any residual period/sequence effects will be examined in the mixed-effects model specified in the SAP [1,2].

- Standardization of conduct:

- Uniform fasting, posture, activity restrictions, and synchronized sampling relative to time zero are enforced across periods to limit performance variability that could differentially influence Cmax and AUC by treatment or period, consistent with FDA and EMA guidance on BA study conduct [1,2].

- Transparent reporting:

- Participant flow, enrollment allocation, and protocol deviations affecting PK evaluability will be reported per CONSORT recommendations for randomized trials to ensure reproducibility and auditability of allocation and analysis decisions [4].

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6.4 Study Intervention Compliance

Oversight, supervision, and subject instruction

- All dosing is conducted under continuous, in-person supervision in the phase 1 clinical pharmacology unit. Qualified staff directly observe identity confirmation, protocol-restricted pre-dose conditions, dose preparation, administration, and immediate post-dose behaviors to ensure the assigned intervention is received as specified for each period and sequence [1–3].
- Prior to each administration, staff reconfirm eligibility and restrictions, provide standardized verbal instructions for sublingual residence (minimize talking/swallowing for 15 minutes) and for posture/activity, and document adherence contemporaneously in source records to support protocol compliance and PK interpretability [1–3].

Verification of correct dosing and full delivery

- Two-person verification: Immediately before administration, a two-person check confirms subject identity, period/sequence assignment, treatment (low-dose sublingual, high-dose sublingual, or IV), dose/volume, and route. Verification is documented in dosing logs and source records with time stamps and signatures/initials [1–3].
- Direct observation: Trained staff witness the entire administration. For sublingual dosing, staff confirm visible deposition of 50 µL or 100 µL under the tongue and absence of immediate expectoration; for IV dosing, staff confirm cannula patency and completion of the 2.5 mL bolus. Start/stop (or end-of-deposition) times define time zero and are recorded to the highest feasible resolution using a synchronized time source [1,2,4].
- Source documentation and eSource integrity: Predose confirmations, dosing details, contemporaneous observations, and timing are recorded as source with ALCOA+ attributes and entered into eSource/eCRF systems with audit trails per FDA electronic source data guidance [3,4].

Dosing logs, accountability, and reconciliation

- Dosing logs capture the administered product, lot/expiry, prepared/dispensed quantities, dose/volume, route, date/time, preparer/verifier, and administering staff. Logs are reconciled with pharmacy accountability records to confirm that quantities dispensed match quantities administered or wasted, ensuring dose fidelity across periods [1,3].
- Chain of custody from preparation through administration and return/waste is maintained and reconciled at predefined intervals and at study closeout. All records are retained to support regulatory inspection and analysis traceability [3,5,6].

Documentation and management of missed, incomplete, or incorrect dosing

- Missed doses: If a scheduled period dose is not administered, the reason and timing are documented in source and the eCRF; the period is excluded from PK analyses for that subject. Rescheduling preserves the protocol-specified washout; all changes are time-stamped and justified per GCP [1–3].

- Incomplete or uncertain delivery:

- Sublingual: Any coughing, drooling, swallowing, or suspected loss within the first minutes postdose is documented with an estimate of loss (none/minimal/moderate/substantial). No re-dosing occurs within the same period. Such periods are typically excluded from the PK per-protocol set due to uncertain delivered dose and may be included in sensitivity analyses as prespecified in the SAP [1,2].
- Intravenous: Any infiltration/extravasation, line occlusion, or early disconnection is recorded with start/stop times and estimated delivered volume. If full delivery is uncertain, the period is excluded from the PK per-protocol set and retained for safety analyses [1–3].

- Incorrect product, route, dose, or timing: Any administration error (e.g., wrong sequence treatment, incorrect volume, major timing deviation) is documented as a protocol deviation with root-cause assessment and corrective/preventive actions. Impact on PK interpretability and analysis set inclusion is determined prospectively and documented prior to database lock, consistent with FDA/EMA bioavailability conduct expectations and ICH-GCP [1–3].

Assessment of compliance impact on PK data

- The investigator and blinded statistician assess whether any dosing noncompliance or postdose behavioral deviation is likely to materially affect absorption or disposition (especially within the early absorption window). Consistent with regulatory guidance, periods affected by early emesis or behaviors expected to alter Cmax/tmax may be excluded from the PK per-protocol set, while retained in PK full analysis and safety sets as appropriate; all rules are prespecified in the SAP and applied prior to unmasking analysis labels [1,2].
- Actual dosing and sampling times recorded in source are used for noncompartmental PK analyses; deviations are handled per SAP with transparent documentation to preserve analysis validity and reproducibility [1,2,4].

Data capture, retention, and monitoring

- Compliance-related data (observational notes, dosing logs, time stamps, accountability/reconciliation records, and deviation reports) are maintained as source documents and captured in the eCRF to support data integrity, regulatory review, and audit. Records are retained per ICH-GCP and U.S. regulations governing BA/BE investigations and investigator recordkeeping [3,5,6].
- Monitors verify concordance among source, eCRF, and accountability records and confirm that any noncompliance is documented with impact assessments and SAP-consistent analysis set assignments, in line with GCP quality requirements [1,3].

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6.5 Dose Modification or Interruption

Fixed single-dose administration and absence of planned modifications

- All study interventions are administered as single, fixed doses per period (0.5 mg sublingual, 1.0 mg sublingual, or 1.0 mg IV) with no planned dose adjustments, titrations, or repeat dosing within a period. This approach is consistent with regulatory expectations for standardized dosing in bioavailability studies to minimize variability and preserve interpretability of primary pharmacokinetic (PK) endpoints [1,2].

Circumstances requiring temporary interruption or discontinuation

- Dosing is a single event per period; therefore, “interruption” applies to events that prevent full delivery of the intended dose or compel cessation of administration in progress. The following circumstances may necessitate withholding, interrupting, or discontinuing dosing for a period and/or further periods for that subject:

- Emergent safety concerns or clinically significant adverse reactions suggestive of excessive antimuscarinic effects (e.g., marked tachycardia, symptomatic hypertension, acute urinary retention, severe xerostomia with dysphagia, acute angle-closure symptoms), or other acute medical events requiring clinical management [3–5].
- Suspected hypersensitivity to atropine or excipients [4,5].
- Dosing errors or product/route mismatch detected prior to or during administration [1–3].
- Incomplete or uncertain dose delivery due to sublingual loss (coughing, drooling, expectoration immediately postdose) or intravenous infiltration/extravasation [1,2,4].
- Discovery of new exclusionary medical information or loss of eligibility immediately prior to dosing (e.g., abnormal vitals/ECG/labs, positive pregnancy test), or noncompliance with critical pre-dose restrictions that may materially affect PK [1–3].

- If clinical risk is identified after a period dose, subsequent periods for that subject may be discontinued. The investigator may also suspend site dosing pending safety review if an unexpected serious risk potentially related to the investigational products is identified [3,6].

Decision authority, documentation, and reporting

- Authority to interrupt or discontinue:

- The principal investigator (PI) or qualified physician sub-investigator has primary authority to withhold, interrupt, or discontinue dosing for subject safety. In an acute situation, any qualified study clinician may immediately halt dosing to protect the subject, with prompt notification to the PI. The PI may consult the sponsor’s medical monitor before resumption of subsequent periods [3].

- Documentation:

- All decisions and their rationales are contemporaneously documented in source records and the electronic case report form (eCRF), including clinical observations, vital signs/ECG, timing relative to dosing, estimate of dose delivered (if incomplete), and any corrective actions. Dosing issues are recorded as protocol deviations with root-cause analysis and preventive measures, consistent with ICH-GCP [1–3].

- Safety reporting:

- Adverse events (AEs) and serious adverse events (SAEs) are captured from dosing onward for each period. SAEs are reported immediately to the sponsor in accordance with ICH-GCP; the sponsor evaluates reportability to regulatory authorities under IND safety reporting requirements (21 CFR 312.32) [3,6]. Any medically significant anticholinergic reactions are managed per clinical judgment

and product labeling, with expedited reporting as required [4,5].

Operational handling of specific dosing contingencies

- Sublingual administration:
  - If coughing, drooling, or expectoration occurs within the first minutes postdose suggesting loss of study drug, no re-dosing occurs in that period. The event, approximate loss (none/minimal/moderate/substantial), and timing are recorded. If vomiting occurs at or before two times the median tmax for the sublingual treatment, the affected period is typically considered non-evaluable for per-protocol PK, consistent with EMA guidance [2].
- Intravenous administration:
  - If infiltration/extravasation or line failure results in uncertain full delivery of the 1.0 mg bolus, administration is stopped and managed clinically. No replacement dose is given in the same period. Start/stop times and estimated delivered volume are documented [1,2,4].
- Dosing errors detected pre-administration:
  - If discovered prior to administration, dosing is withheld, the error is documented, and dosing may be rescheduled provided protocol prerequisites are re-established and the crossover washout is preserved. If an error is detected during administration, dosing is halted and managed as above; the period is not repeated within the same scheduled session [1–3].

Reporting of interrupted or discontinued dosing

- Any period with interrupted or incomplete dosing is:
  - Recorded in source/eCRF with precise timing, estimated delivered fraction (if estimable), and clinical course.
  - Reported as a protocol deviation when applicable, cross-referenced to AE/SAE entries if clinically driven.
  - Communicated to the sponsor/monitor according to site and study reporting procedures to enable timely safety and data quality review in alignment with ICH-GCP [1,3,6].

Consequences for PK analysis sets and data handling

- General rules:
  - Actual dosing and sampling times are used for PK analyses. Periods with incomplete or uncertain dose delivery, early emesis relevant to absorption, major timing deviations, or noncompliance likely to affect absorption/disposition are excluded from the PK per-protocol set (PK-PPS) and retained in the PK full analysis set (PK-FAS) and safety set, per regulatory guidance for BA studies [1,2].
- Sublingual-specific:
  - Suspected early loss of sublingual dose or vomiting at or before two times the median tmax generally renders the period non-evaluable for PK-PPS; sensitivity analyses may be performed per the statistical analysis plan (SAP) [2].
- Intravenous-specific:
  - Uncertain completion of the IV bolus (e.g., infiltration) results in exclusion from PK-PPS due to unknown actual dose; if sufficient concentrations exist to inform safety, the period remains in safety analyses [1,2,4].
- Truncated concentration–time profiles:
  - If an AE necessitates early discontinuation of sampling but the dose was fully delivered, available data are analyzed using noncompartmental methods with appropriate censoring; AUC extrapolation is assessed for reliability per SAP. Inclusion in PK-PPS depends on prespecified completeness criteria consistent with FDA/EMA expectations [1,2].

Resumption criteria for subsequent periods

- A subject with a dosing interruption or clinically significant AE may resume subsequent periods only if:

- The event has resolved to baseline or acceptable clinical status, the PI deems re-exposure safe, and all protocol eligibility and pre-dose conditions are reconfirmed. If risk remains unacceptable, the subject discontinues from further dosing. These determinations and their justifications are documented per ICH-GCP [1,3].

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6.6 Continued Access After Study

Policy on post-trial access

No continued access to the investigational administration of atropine sulfate via the sublingual route will be provided after a subject completes or withdraws from this study. This is a single-dose, non-therapeutic bioavailability investigation in healthy volunteers without an underlying medical condition requiring ongoing atropine therapy, and there is no clinical indication for continued dosing beyond protocol procedures [1]. Both study products are FDA-approved and commercially available for clinical use (atropine sulfate injection 0.4 mg/mL and atropine sulfate ophthalmic solution USP 1%); any future medical need for atropine will be managed under routine care outside the trial [2,3]. Off-label use of approved products, including potential sublingual administration of the ophthalmic solution, falls within the practice of medicine at the discretion of licensed clinicians and is outside the scope and obligations of this research protocol [4].

Rationale and ethical/regulatory alignment

- The Declaration of Helsinki frames post-trial access around continued provision of proven beneficial interventions needed by participants after study completion; this provision does not apply to a one-time pharmacokinetic study in healthy volunteers without a therapeutic objective [1].
- FDA’s expanded access mechanisms address treatment use of investigational drugs for patients with serious or immediately life-threatening conditions lacking satisfactory alternatives and are not applicable to healthy volunteer pharmacology studies [5].
- Investigator responsibilities for subject safety continue after dosing as clinically indicated: the investigator will ensure appropriate medical care and follow-up for any adverse events related to study participation until resolution or stabilization, with referral as needed, consistent with ICH-GCP [6].

Responsibilities during the post-study period

- No open-label extension or post-trial supply is planned or offered.
- The site will not dispense atropine for non-study use. Any subsequent clinical use, if indicated, will occur under standard medical care using approved products procured through routine channels [2,3].
- The investigator will arrange or provide appropriate follow-up for adverse events attributable to study participation and document outcomes per GCP [6].

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6.7 Treatment of Overdose

Definition and likelihood

An overdose is defined, for the purposes of this protocol, as any unintended atropine exposure in a study participant that exceeds the assigned protocol dose for the period, involves an unintended additional dose or incorrect route resulting in higher-than-intended systemic exposure, or produces a clinical syndrome consistent with antimuscarinic toxicity requiring medical intervention, irrespective of the known administered amount. Given single, fixed, supervised dosing with two-person verification and inpatient observation, overdose is unlikely under controlled Phase 1 unit conditions [1].

Clinical features of atropine toxicity

Atropine overdose may present with dose-related antimuscarinic manifestations including tachycardia, hypertension, hyperthermia, flushed/dry skin, mydriasis with blurred vision, decreased bowel sounds/ileus, urinary retention, xerostomia, agitation, delirium, and, in severe cases, seizures, arrhythmias, and coma [2–4]. The approved labeling for atropine injection and ophthalmic atropine describes these systemic effects and warns of potentially life-threatening complications with excessive dosing [3,4].

Immediate actions and on-site evaluation

- Stop administration immediately if in progress; secure airway, breathing, and circulation. Place the subject on continuous cardiac, pulse oximetry, and noninvasive blood pressure monitoring; obtain temperature and capillary blood glucose [2–4].

- Establish IV access, obtain a 12-lead ECG, and perform focused neurologic and mental status examinations. Initiate active external cooling for hyperthermia and provide supplemental oxygen as indicated [2].
- Notify the principal investigator (PI) or on-call study physician without delay; mobilize the site emergency response per institutional SOPs. Document time of symptom onset, product, lot, route, intended and estimated administered dose/volume, and concurrent circumstances [1].

Supportive care and specific treatment

- General supportive care is the mainstay: titrate isotonic IV fluids for hypotension, treat agitation and seizures with benzodiazepines (e.g., lorazepam), avoid antipsychotics that may worsen anticholinergic effects, manage urinary retention (e.g., bladder scan and catheterization if needed), and initiate active cooling for hyperthermia [2].
- Physostigmine may be considered for severe anticholinergic delirium or agitation that is refractory to benzodiazepines, provided there are no contraindications such as suspected tricyclic antidepressant co-exposure, significant QRS/QT prolongation, or seizure predisposition; dosing and cardiac monitoring should follow medical toxicology guidance, with readiness to manage cholinergic adverse effects [2]. This reflects interpretive, consensus-based application of toxicology practice to atropine toxicity supported by peer-reviewed summaries [2].
- Gastrointestinal decontamination is generally not indicated in this setting due to small study volumes and predominantly sublingual or intravenous administration; if a large inadvertent oral ingestion were to occur and presentation is within 1 hour, single-dose activated charcoal may be considered after airway protection assessment [2].

Criteria for escalation and transfer

Prompt transfer to an acute care hospital (via emergency medical services) is warranted for any of the following:

- Persistent or worsening altered mental status/delirium, seizures, or psychosis despite initial benzodiazepines [2].
- Hemodynamic instability, symptomatic tachyarrhythmia, chest pain, syncope, or new ischemic changes/arrhythmias on ECG [2–4].
- Hyperthermia (core temperature ≥38.5–39.0°C) or rhabdomyolysis concerns [2].
- Refractory urinary retention, severe ileus, or any condition requiring higher-level monitoring or interventions not available on site [2].

The study physician coordinates transfer and provides a detailed handover including administered dose(s), timing, clinical course, treatments given (including any physostigmine), and relevant study contact information.

Monitoring, follow-up, and documentation

- Continuous cardiorespiratory and neurologic monitoring continues until clinical stabilization. Serial vital signs, ECGs, and focused exams are documented at intervals appropriate to severity; laboratory tests (e.g., electrolytes, renal function, creatine kinase) are obtained as clinically indicated [2–4].
- The PI or qualified physician sub-investigator is responsible for clinical assessment, orders, and disposition. All findings, interventions, and outcomes are contemporaneously recorded in source documents and the eCRF per ICH-GCP [1].
- Participants with overdose events are followed until resolution or stabilization, with outcomes and any sequelae documented.

Reporting requirements

- Any overdose is recorded as an adverse event (AE) and assessed for seriousness, severity, causality, and outcome. Serious adverse events (SAEs), including life-threatening manifestations or hospitalization, are reported to the sponsor immediately in accordance with ICH-GCP, and the sponsor evaluates reportability to regulatory authorities under IND safety reporting requirements (21 CFR 312.32) [1,5].
- Overdose events are documented with complete medication error details (if applicable), root-cause analysis, and corrective/preventive actions. All related correspondence and reports are retained in the trial master file and investigator site file per GCP [1].

Roles and responsibilities

- The PI or designated study physician leads medical evaluation and management decisions, including hospital transfer. Registered nurses and other qualified clinical staff perform immediate supportive measures within their scope, implement monitoring, and assist with documentation.
- The PI ensures timely notification to the sponsor and, when required by local regulations or IRB policy, to the IRB/IEC; the sponsor's medical monitor remains available for consultation during management and follow-up [1,5].

References

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6.8 Concomitant Medications

General policy and time window

Concomitant medications, including prescription drugs, over-the-counter (OTC) products, herbal preparations, and dietary supplements, are prohibited from 30 days prior to Period 1 dosing through the final pharmacokinetic (PK) sample of Period 3 unless medically necessary and approved by the investigator. This restriction minimizes pharmacokinetic and pharmacodynamic confounding in bioavailability studies and aligns with regulatory expectations to control factors that may influence absorption, metabolism, or elimination of the investigational product [1,2]. All concomitant medication use will be documented per Good Clinical Practice (GCP) requirements [3].

Prohibited medications and products

The following categories are prohibited due to potential to alter atropine exposure, interfere with sublingual absorption, confound safety assessments, or otherwise affect study interpretability;

examples are illustrative and not exhaustive:

- Enzyme/transporter modulators: strong or moderate inducers or inhibitors of drug-metabolizing enzymes or transporters (e.g., potent CYP3A inducers such as rifampin; potent CYP3A inhibitors such as itraconazole; P-gp modulators), given the potential to alter systemic exposure and increase inter-subject variability in BA studies [1,2,7].
- Agents that alter salivary flow, oral mucosa, or sublingual residence: anticholinergic or sympathomimetic drugs that reduce salivation (e.g., scopolamine, first-generation H1 antihistamines, tricyclic antidepressants), sialogogues that increase salivation (e.g., pilocarpine), topical oral agents with high ethanol content, or mucosal anesthetics; saliva volume and mucosal conditions can significantly influence sublingual drug dissolution, residence time, and membrane permeability [2,8].
- Drugs affecting gastrointestinal motility or secretions: prokinetics (e.g., metoclopramide), antidiarrheals with anticholinergic effects, and antacids with high cation load; such agents may confound tolerability signals and systemic handling of atropine and are routinely restricted in BA investigations [1,2].
- Pharmacodynamic interactors with antimuscarinic effects: other anticholinergics (e.g., oxybutynin, tolterodine), amantadine, certain antipsychotics, and first-generation antihistamines, which can potentiate atropine's adverse effects [4,5,6].
- Cholinesterase inhibitors: donepezil, rivastigmine, galantamine, pyridostigmine; these may antagonize atropine's effects and alter clinical responses [4,5,6].
- Drugs that materially affect heart rate, atrioventricular conduction, or blood pressure: beta-blockers, certain calcium channel blockers (non-DHP), class I/III antiarrhythmics; these may confound safety monitoring and atropine's chronotropic effects [4,5].
- Alkalinizing or acidifying agents that can alter urinary excretion of weak bases: chronic sodium bicarbonate or acetazolamide (alkalinization) and agents producing marked acid–base shifts; urinary pH can influence renal elimination of atropine and related compounds, potentially affecting exposure metrics [6].
- Centrally active sedatives/hypnotics and recreational substances: benzodiazepines, Z-drugs, cannabinoids, and other CNS depressants; these may confound neurologic safety assessments and are incompatible with Phase 1 unit restrictions [1,2].
- Herbal products and supplements: St John's wort (induction), grapefruit-derived products (inhibition), and any supplement with anticholinergic or sympathomimetic properties; herbal products are heterogeneous and commonly excluded to reduce variability [1,2,7].

If a prohibited product is inadvertently taken, the investigator will assess impact on PK interpretability; affected periods may be excluded from the PK per-protocol set per prespecified SAP rules, consistent with BA guidance [1,2].

Permitted medications under defined conditions

- Rescue analgesia: Acetaminophen up to 1,000 mg per 24 hours may be permitted for intercurrent pain or post-procedural discomfort, avoiding the interval from 24 hours pre-dose to completion of the last PK sample in each period; use outside this window requires investigator approval. This allowance reflects protocol-specific, interpretive judgment to enable subject comfort without expected impact on atropine PK; all use will be recorded contemporaneously.
- Hormonal contraception: Ongoing, stable-dose hormonal contraceptives are permitted and should be maintained unchanged throughout the study; use is documented to support safety oversight. This reflects interpretive, protocol-specific judgment that no clinically meaningful interaction with atropine PK is expected based on pharmacology and labeling [4,5].

No other concomitant medications are permitted during confinement periods unless deemed medically necessary by the investigator.

Medically necessary medications: approval and management

- Circumstances: Treatment of acute intercurrent illness or clinically significant adverse events may require non-protocol medications. The investigator (or qualified physician designee) may authorize use when the anticipated clinical benefit outweighs potential effects on PK or safety assessments [3].
- Approval process: Prior approval by the principal investigator (PI) or sub-investigator is required whenever feasible; urgent care follows standard medical practice with notification to the PI at the earliest opportunity. The sponsor medical monitor should be consulted for decisions that could affect PK evaluability or require period rescheduling [1–3].
- Washouts and scheduling: When possible, dosing will be postponed to allow an appropriate washout for short-course necessary medications, consistent with their known half-lives and interaction potential per FDA DDI principles [7]. Any rescheduling will preserve the protocol-specified washout between study treatments [1,2].

Documentation requirements

All concomitant medications taken from screening through study completion will be recorded in source documents and the eCRF with the following details: generic name; indication; route; dose, unit, and frequency; start and stop dates/times; and timing relative to each period's dosing. Investigators will assess and document whether each medication is prohibited, permitted, or medically necessary, and its potential impact on PK and safety assessments, in accordance with ICH-GCP [3].

Data review and assessment of impact on PK

Concomitant medication data will be reviewed prior to database lock. Periods in which prohibited or impactful medications were used in proximity to dosing may be excluded from the PK per-protocol set and retained in sensitivity or safety analyses, per prespecified SAP rules and regulatory guidance for BA studies [1,2]. The blinded statistician will evaluate temporal proximity, mechanism (e.g., enzyme modulation, effects on saliva/oral mucosa), and plausibility of PK impact using authoritative DDI information when applicable [7]. Any exclusions or analysis-set changes will be prospectively documented.

References

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2. European Medicines Agency (EMA). Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**). London: EMA; 2010. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf
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6.8.1 Rescue Medications

Policy and intent

Rescue medications are permitted only when clinically necessary to manage emergent adverse events (AEs) or intercurrent conditions and must be selected to avoid pharmacokinetic (PK) interference and confounding of safety assessments in this single-dose, healthy-volunteer bioavailability study [1–3]. Non-pharmacologic measures are preferred whenever adequate. All use of rescue medications will be aligned with the study's safety oversight and adverse event management procedures and documented to preserve PK interpretability [1–3].

Anticipated symptomatic needs and preferred management

- Common antimuscarinic effects (e.g., xerostomia, transient blurred vision, mild tachycardia, lightheadedness) will be managed primarily with non-pharmacologic measures: rest in supine or semi-recumbent position, reassurance, environmental cooling, and small sips of water permitted beginning 60 minutes postdose per lifestyle restrictions [4,5]. Early aggressive oral fluid intake is avoided before 60 minutes to protect sublingual absorption assessments [1,2].
- For urinary hesitancy/retention, non-drug strategies (privacy, time, bladder scan, and if needed, catheterization) are preferred over pharmacologic reversal agents to avoid interference with atropine's pharmacology and PK [4,5].
- Ocular discomfort from mydriasis is managed with reduced ambient light; miotic agents are not planned and would be used only if clinically imperative [4,5].

Allowed rescue agents, indications, dose limits, and timing relative to PK

Rescue pharmacotherapy is restricted during the early absorption and distribution windows to minimize potential PK confounding. Unless immediate medical need dictates otherwise, avoid rescue medications until after the 2-hour sample; stricter restrictions are noted below. If urgent care is required earlier, prioritize agents with minimal likelihood to alter atropine plasma concentrations, and document exact timing relative to dosing and samples [1,2]. The table summarizes permitted options.

Clinical circumstance	Preferred rescue agent(s)	Typical adult dose limits	Timing safeguards relative to PK sampling
Procedural or intercurrent pain (e.g., venipuncture discomfort, headache)	Acetaminophen (paracetamol) oral	Up to 1,000 mg per 24 h	Avoid from 24 h predose until completion of the last PK sample in the period; if medically necessary earlier, defer until after 2 h postdose whenever feasible; record exact time [1–3]
Nausea/vomiting (not expected)	Ondansetron oral/IV	4 mg once; may repeat per standard care if needed	Prefer administration after 2 h postdose; if given earlier for urgent care, document timing to inform PK set inclusion per SAP [1–3]
Symptomatic tachycardia with anxiety or agitation (clinically significant)	Non-pharmacologic measures; short-acting benzodiazepine (e.g., lorazepam) if severe agitation/anxiety	Lorazepam 0.5–1 mg PO/SL/IV, single dose	Use only if clinically indicated; avoid before 2 h postdose unless necessary for safety; continuous monitoring and documentation required [2–4]
Hyperthermia or heat discomfort	External cooling, IV isotonic fluids if clinically indicated	Per standard care	May be used at any time if medically necessary; fluids do not affect atropine PK; document rates and volumes [2,4]
Urinary retention	Bladder scan; intermittent catheterization	Non-drug	May be used at any time; avoid pharmacologic cholinergic

			agonists to prevent PK/PD confounding [4,5]
Severe anticholinergic delirium or life-threatening toxicity (unlikely)	Physostigmine with continuous ECG monitoring (per toxicology practice)	As per institutional protocol and toxicology guidance	Use only for severe, refractory cases under physician supervision; timing and dosing rigorously documented; safety procedures per overdose management apply [2,4]

Interpretive note: The above selections reflect protocol-specific, conservative choices to enable subject safety while minimizing effects on atropine PK. Agents with anticholinergic or cholinergic properties (e.g., diphenhydramine, scopolamine, pilocarpine) are not permitted as rescue due to pharmacodynamic interaction potential unless deemed essential for immediate medical care, in which case full documentation applies [4,5].

Prohibited rescue agents (unless life-saving)

- Antimuscarinic or cholinergic agents (e.g., scopolamine, diphenhydramine, oxybutynin, pilocarpine), gastrointestinal motility modifiers (e.g., metoclopramide), and sialogogues are prohibited because they may alter atropine's pharmacology or PK or confound tolerability endpoints. Life-saving exceptions follow physician judgment; all such use triggers PK per-protocol set review per the Statistical Analysis Plan (SAP) [1,2,4,5].
- Drugs known as strong enzyme/transporter modulators are prohibited as rescue due to confounding risk in BA studies [1,2].

Timing principles to protect PK interpretability

- Critical window: From 1 hour predose through 2 hours postdose, avoid administering rescue medications unless required for immediate safety. This window covers sublingual dissolution/absorption and early distribution phases and the majority of early PK sampling (up to 120 minutes) [1,2].
- If rescue medication is administered within the critical window, record actual dosing and sampling times to the minute; affected periods may be excluded from the PK per-protocol set if the medication plausibly impacts absorption or disposition, consistent with FDA/EMA guidance and prespecified SAP rules [1,2].
- Non-pharmacologic measures may be initiated at any time as clinically indicated.

Documentation and linkage to safety oversight

- Source records: Record generic name, indication, dose, route, lot (if available), start/stop dates and times, and timing relative to index dose and each PK sample. Capture clinical rationale, response, and any monitoring (e.g., ECG) [3].
- eCRF: Enter all rescue medications as concomitant medications with linkage to the precipitating AE. Classify whether use occurred within the critical window and whether it is prohibited, permitted, or life-saving exception per protocol [1–3].
- Safety alignment: Management follows the study's adverse event management and escalation procedures; serious or medically significant events are reported per ICH-GCP and applicable regulations. Rescue treatment does not modify the obligation to follow overdose and emergency procedures when indicated [3–5].
- PK analysis impact: Prior to database lock and while blinded to sequence labels, the investigator and blinded statistician will assess the potential for PK interference and assign analysis set inclusion per SAP. All decisions will be traceably documented in the data review meeting minutes and analysis set flags [1,2].

References

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7 DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT WITHDRAWAL

7.1 Criteria for Discontinuation of Study Intervention

General principles

Discontinuation of study intervention for an individual subject will occur when predefined safety or data integrity conditions are met. Decisions are intended to protect participant safety and preserve the interpretability of pharmacokinetic (PK) and safety data and will be made by the investigator and/or designated medical monitor based on clinical judgment within these protocol-defined criteria [1–3].

Safety-based discontinuation

Administration of the investigational or reference product will be stopped or not initiated for any of the following:

- Any serious adverse event (SAE), medically significant adverse event (AE), or clinically significant deterioration in vital signs, physical examination, 12-lead ECG, or laboratory parameters that, in the investigator’s judgment, raises a safety concern with further exposure (e.g., symptomatic tachyarrhythmia, sustained hypertension, hyperthermia, acute confusional state, or other manifestations consistent with antimuscarinic toxicity) [2,4,5].
- Hypersensitivity or suspected allergic reaction to atropine or excipients, or evidence of acute angle-closure glaucoma precipitated by mydriasis [4,5].
- Pregnancy or positive pregnancy test at any time prior to dosing or during the study in a woman of childbearing potential [2].
- Intercurrent illness or new medication requirement that materially increases risk with atropine exposure (e.g., initiation of potent anticholinergic or cholinergic agents, strong enzyme/transporter modulators) per investigator assessment [1–3].
- Any overdose, medication error, or dosing route error; further dosing is halted for that period pending safety evaluation [2].

Safety-based discontinuations and interruptions will be documented in source records and the electronic case report form (eCRF); AEs/SAEs will be reported in accordance with ICH-GCP and applicable regulations, including expedited IND safety reporting criteria when met [2,6].

Vomiting/emesis after dosing

- Definition and window for PK impact: Vomiting that occurs during the early absorption phase can invalidate PK assessment. Consistent with regulatory guidance, if vomiting occurs within a window up to twice the median tmax for the administered treatment, the affected period's PK data will be excluded from the per-protocol PK analysis set unless justified otherwise a priori in the Statistical Analysis Plan (SAP) [1,3]. This rule will be applied using the treatment-specific median tmax defined for this study or established literature, with documentation of onset time relative to dosing and PK samples [1,3].
- Operational handling:

- If vomiting occurs within the defined window after sublingual dosing, dosing for that period is considered complete; no immediate re-dosing will occur in the same confinement period. The period may be repeated in an additional scheduled period after the protocol-specified washout if the investigator deems re-exposure safe and the subject remains eligible; otherwise the subject may continue in remaining periods if safe and feasible [1–3].
- If vomiting occurs after the defined window, PK data from that period may be retained, provided other protocol conditions are met and per SAP rules [1,3].
- For intravenous dosing, vomiting does not affect dose delivery but indicates an AE; inclusion of PK data follows the same window-based rule for consistency, with final inclusion per SAP and blinded data review [1,3].

All emesis events will be recorded as AEs with exact timing; related management (e.g., antiemetics) will be captured to inform PK evaluability [2,3].

Protocol deviations compromising data reliability

Dosing will be withheld, interrupted, or the period discontinued when critical protocol deviations occur that would compromise PK interpretability, including:

- Fasting status not met (food or liquid intake within 1 hour predose or 1 hour postdose contrary to protocol) [1,3].
- Missed, partial, or incorrect dosing (e.g., incomplete sublingual administration, spillage, incorrect volume, dosing outside the allowed window, or IV extravasation) [1–3].
- Major PK sampling deviations (e.g., missed early samples, sample timing errors that preclude reliable AUC/Cmax estimation, hemolyzed or otherwise unusable key samples) [1,3].
- Use of prohibited concomitant medications or rescue treatments within the critical absorption window with plausible impact on atropine PK or pharmacodynamics, as prespecified in the SAP [1,3].
- Randomization or sequence assignment errors affecting treatment administration [1,3].

In such cases, the period's dosing may be cancelled prior to administration or considered completed with exclusion from the per-protocol PK set after administration; the investigator may reschedule the period after an appropriate washout when feasible and safe. All deviations are recorded in source and eCRF with root-cause assessment and impact on analysis sets per regulatory guidance [1–3].

Withdrawal of informed consent

If a subject withdraws informed consent at any time prior to completion of the study intervention for a given period, dosing for that period will be discontinued immediately. With the subject's permission, safety follow-up will be conducted and any already-collected data retained as allowed by regulations and the consent form [2].

Authority, documentation, and reporting

- Authority: The investigator and/or designated medical monitor has authority to interrupt or discontinue study intervention based on clinical judgment and these protocol criteria; decisions prioritize subject welfare and data integrity [2].
- Documentation: Each decision to withhold, interrupt, or discontinue dosing will be contemporaneously documented in source records and the eCRF, including rationale, timing, related clinical findings, actions taken, and implications for PK analysis set assignment per SAP [1–3].
- Reporting: All AEs/SAEs prompting discontinuation will be assessed for seriousness, severity, and causality and reported per ICH-GCP and applicable regulations, including 21 CFR 312.32 for IND safety reporting when criteria are met. Protocol deviations affecting subject safety or data integrity will be reported per sponsor and institutional policies and retained in the trial master file and investigator site file [2,6].

These discontinuation criteria are expressly designed to protect participant safety and to preserve the validity and reliability of the PK and safety assessments central to this bioavailability study [1–3].

References

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7.2 Criteria for Withdrawal of Participant from Study

Withdrawal criteria

A participant will be withdrawn from further study participation under any of the following circumstances, with decisions made by the investigator in alignment with Good Clinical Practice and applicable regulations [1]:

- Voluntary withdrawal of informed consent by the participant at any time, with or without reason [1,2].
- Occurrence of a clinically significant adverse event (AE) or any serious adverse event (SAE) for which, in the investigator's judgment, continued participation is not in the participant's best interest (e.g., symptomatic tachyarrhythmia, sustained hypertension, hyperthermia, acute confusional state, hypersensitivity) [1].
- Investigator determination, based on clinical judgment or new information, that continued participation poses unacceptable risk or is otherwise not in the participant's best interest (e.g., new illness, new clinically significant ECG or laboratory abnormality, or initiation of a prohibited medication) [1].
- Pregnancy or positive pregnancy test at any time during the study in a woman of childbearing potential [1].

- Noncompliance with study procedures, restrictions, or visit schedules that, in the investigator's judgment, compromises safety or data integrity (e.g., prohibited concomitant medication use, failure to maintain fasting requirements around dosing) [1,3,4].
- Missed visits or repeated missed PK samples that preclude valid assessment of primary endpoints or completion of crossover periods, as determined by the investigator in consultation with the blinded statistician per the Statistical Analysis Plan (SAP) [3,4].
- Administrative or sponsor decisions, including early termination of the study or site, protocol amendments that render the participant ineligible, or regulatory authority/IRB-IEC actions requiring cessation [1].
- Any overdose, medication error, or dosing route error where continued participation is considered unsafe or scientifically noninformative, per investigator judgment [1].

Communication and documentation of withdrawal

Participants may communicate a decision to withdraw consent verbally or in writing to any study staff member. The date/time of withdrawal and reason (if provided) will be recorded in source documents and the electronic case report form (eCRF); participants will be reminded that providing a reason is optional [1,2]. The investigator will document the specific withdrawal criterion met, actions taken, and any related AEs/SAEs, ensuring contemporaneous, attributable, legible, and complete records per ICH-GCP [1].

Post-withdrawal safety evaluations

When feasible and acceptable to the participant, an early termination visit will be performed to protect safety and meet reporting obligations [1]:

- Interval medical history and concomitant medication review; targeted AE/SAE assessment.
- Vital signs and focused physical exam; 12-lead ECG.
- Clinical laboratory tests as indicated by preceding AEs or clinically significant findings.
- Pregnancy testing for women of childbearing potential when appropriate.

Participants withdrawn for safety reasons (including overdose or pregnancy) will be followed until resolution or stabilization of AEs/SAEs, consistent with ICH-GCP and regulatory reporting requirements [1,5]. If a participant withdraws consent for all further contact or procedures, no additional clinical evaluations will be performed; safety reporting will rely on information already obtained, with retention of previously collected data as described below [2].

Handling of data after withdrawal

- Data retention: Data collected up to the time of withdrawal will be retained and included in the study database and analyses as necessary to preserve scientific integrity and meet regulatory obligations, in accordance with FDA guidance and ICH-GCP. No new data will be collected after complete consent withdrawal except safety information necessary to fulfill legal or regulatory reporting obligations or procedures to which the participant separately consents [1,2].
- PK and endpoint analyses: Available PK samples and assessments obtained prior to withdrawal will be used to the extent appropriate. Partial PK profiles may contribute to Cmax and observed concentrations and, when sufficient time–concentration data exist, to AUC0–t; AUC0–∞ and t½ will be estimated only when data support reliable extrapolation, per SAP rules. Per FDA and EMA guidance for bioavailability/bioequivalence studies, periods with incomplete or nonevaluable PK (e.g., critical early samples missing) may be excluded from the per-protocol PK set and retained in supportive or safety analyses following prespecified criteria and blinded review [3,4]. Interpretive synthesis: Final inclusion/exclusion of partial data will be determined by the blinded statistician and investigator based on data sufficiency and plausibility of unbiased parameter estimation, applying the SAP and relevant guidance [3,4].

Replacement of withdrawn participants

To achieve approximately 12 evaluable participants, individuals who withdraw before completing sufficient, evaluable data for the primary PK endpoints may be replaced. Replacement is permitted when:

- Withdrawal occurs before dosing in any period, or
- Withdrawal or nonevaluability (e.g., major protocol deviation, prohibited medication use, missed critical PK samples) in one or more periods renders the participant nonevaluable for primary PK analyses per SAP [3,4].

The principal investigator, in consultation with the sponsor medical monitor and blinded statistician, will determine the need for and number of replacements, consistent with the planned enrollment approach and maintaining allocation balance across sequences [1,3,4].

Notifications and reporting

- Safety-related withdrawals (e.g., due to AE/SAE, overdose, or pregnancy) will be reported to the sponsor immediately and documented as AEs/SAEs with assessment of seriousness, severity, causality, and outcome. The sponsor will determine expedited reporting to regulatory authorities under IND safety reporting (21 CFR 312.32) when criteria are met [1,5].
- The investigator will notify the IRB/IEC per institutional policy when a withdrawal is prompted by a safety concern, pregnancy, or other reportable event, and when an unanticipated problem involving risks to subjects or others is identified [1].
- All withdrawals and their reasons will be summarized in study reports, consistent with ICH-GCP documentation standards [1].

References

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7.3 Lost to Follow-up Procedures

Definition and scope

For this bioavailability crossover study, a participant is considered lost to follow-up (LTFU) when, despite predefined, reasonable, and repeated attempts using consented contact methods, the investigator cannot establish contact or ascertain the participant’s willingness or ability to continue, and the participant fails to attend a scheduled visit or complete essential procedures, with no study-related assessments obtained thereafter during the relevant study period or the remainder of the trial, as applicable. This operational definition aligns with Good Clinical Practice expectations for subject tracking, documentation, and safeguarding welfare during trial conduct [1]. Interpretive synthesis: This

protocol applies a conservative, audit-ready definition to support transparent accounting of participants and minimize missing data, consistent with SPIRIT/CONSORT expectations for planning and reporting follow-up and losses [4,5].

Triggers for LTFU follow-up actions

- Missed or no-show for any scheduled on-site confinement/dosing visit or required postdose assessment without prior notification.
- Failure to respond to site outreach within 24 hours before a scheduled dosing day or within 24 hours after a missed visit.
- Returned undeliverable communications or disconnected phone numbers indicating potential relocation or change in status.

Upon any trigger, the LTFU contact sequence begins immediately and continues until status is ascertained or LTFU is declared per this section [1].

Contact methods, minimum attempts, and timeframes

Interpretive synthesis: To meet GCP standards for reasonable efforts and ensure timely safety oversight in a healthy-volunteer Phase 1 unit, the following standardized outreach algorithm will be implemented [1,4]:

- Approved contact modalities (as consented): telephone calls (mobile and landline), voicemail, text/SMS, email, secure portal/app messaging (if applicable), and postal mail. Contact of the designated emergency contact is permitted solely to request assistance in reaching the participant or to convey safety-critical information, consistent with consent and privacy policies [1].
- Attempt requirements:

- A minimum of six contact attempts over a 14-day window, varying days of week and times of day, using at least three different modalities when available (e.g., two phone calls, one voicemail, two emails/texts, one postal letter) [1,4].
- At least one telephone attempt within 24 hours of the missed visit and at least one attempt during nonbusiness hours to improve reachability [4].
- If no response by Day 7, send a traceable/certified postal letter to the last known address requesting contact within 7 days; retain delivery records in the site file [1].
- If an urgent safety concern exists (e.g., serious AE reported before loss of contact), intensify attempts (e.g., daily calls for 3 consecutive days, expedited postal letter) to fulfill safety follow-up obligations; document rationale [1,3].

- Cessation of attempts: Stop earlier if the participant or a legally authorized representative confirms withdrawal of consent for further contact, or if contact is re-established and a plan is agreed [1,2].

Documentation of all outreach efforts

All attempts will be contemporaneously documented in source records and the eCRF, including:

- Date/time, modality, phone/email/address used, and staff member.
- Outcome (e.g., no answer, voicemail left, message sent, undeliverable, spoke with participant/emergency contact) and any response details.
- Any safety information obtained and actions taken.

Documentation must be attributable, legible, contemporaneous, original, and accurate per ICH-GCP; postal proof-of-mailing/receipt will be filed in the investigator site file/trial master file as applicable [1].

Determination and classification of participant status

Based on outcomes of attempts, the investigator will classify the participant as one of the following and record the classification date in source and eCRF:

- Lost to follow-up: No contact or status ascertainment after completion of the required attempts/timeframe.
- Relocated/continued elsewhere: Participant not returning to the site but confirms relocation or follow-up at another facility without continued participation.
- Withdrawn without notice: Participant explicitly or implicitly indicates discontinuation but declines to complete withdrawal procedures or further contact.

If contact is re-established, the investigator will assess feasibility and safety of continued participation and document the plan; re-consent may be obtained if required by the IRB/IEC for significant interruptions [1].

Data handling for LTFU

All data and biospecimens collected up to the point of LTFU will be retained in the study database and may be included in analyses as appropriate to preserve scientific integrity and comply with regulatory obligations, consistent with FDA guidance on data retention and ICH-GCP [1,2]. No new data will be collected after a participant withdraws consent for further contact/procedures; safety information already known to the investigator remains reportable as required [2,3]. Interpretive synthesis: For pharmacokinetic analyses, partial profiles obtained before LTFU may contribute to parameter estimation per the Statistical Analysis Plan, with inclusion determined by blinded review of data sufficiency and plausibility.

Investigator responsibilities for safety follow-up and reporting

- Reasonable safety follow-up: Investigators must make and document reasonable efforts to ascertain safety outcomes for any participant who becomes unreachable, including attempts to confirm resolution of ongoing AEs/SAEs known at last contact. Any safety-relevant information obtained from participants, emergency contacts, medical records, or other legitimate sources should be recorded and reported per GCP and regulatory requirements [1,3].
- Reporting obligations: Any SAE becoming known to the investigator (e.g., hospitalization or death discovered during outreach or via public notice) will be reported to the sponsor immediately and handled in accordance with IND safety reporting regulations and institutional policies, regardless of LTFU status [1,3].
- Confidentiality: All outreach and documentation will adhere to applicable consent, privacy, and confidentiality requirements, using only consented contact channels and sharing the minimum necessary information to ensure safety and trial conduct compliance [1].

Accounting and reporting of LTFU

The number of participants who are LTFU, along with timing and reasons when known, will be prospectively captured in the eCRF and summarized in the clinical study report, consistent with SPIRIT/CONSORT standards for transparent reporting of participant flow and losses [4,5].

References

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8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Pharmacokinetic (PK) Assessments

Matrices for PK evaluation

- Primary matrix: K2EDTA plasma for quantitation of total atropine concentration and derivation of all primary PK parameters (AUC0–t, AUC0–∞, Cmax, tmax, t½, CL, Vd for IV; CL/F, Vd/F for sublingual) [1–3].
- Exploratory matrix: Urine for characterization of cumulative urinary excretion (Ae0–8) and fraction excreted unchanged (fe0–8) to support interpretation of clearance and absorption; urinary data will be descriptive and supportive only [2–4]. Interpretive synthesis: Given that a clinically meaningful proportion of atropine is excreted unchanged in urine in adults and the elimination half-life is approximately 2–4 hours, short-interval pooled collections through 8 hours are expected to capture the majority of renal elimination after single doses [2–4].

PK sampling schedule and duration

- Blood (plasma) sampling per period: Predose (within 60 minutes prior to dosing), and at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes, and 2, 4, 6, and 8 hours postdose (total 13 postdose samples plus 1 predose; 14 samples/period/subject). Exact sampling times relative to actual dosing will be recorded for all collections [1,3].
- Urine (exploratory) per period: Predose void (–60 to 0 minutes) and pooled intervals of 0–2, 2–4, 4–6, and 6–8 hours postdose; total urine volume measured for each interval, with aliquots retained for analysis [2–4].
- Sampling duration justification: The schedule emphasizes early intensive sampling to define absorption rate and Cmax/tmax for sublingual administration and to profile rapid distribution after IV dosing, followed by extended sampling to at least 8 hours to characterize the terminal log-linear phase. This duration is expected to exceed three elimination half-lives for atropine in healthy adults, enabling reliable estimation of AUC0–∞ with extrapolated fraction typically <20% in accordance with regulatory expectations [1,3,4]. Interpretive synthesis: The inclusion of multiple sub-hourly samples through 60 minutes is consistent with atropine's rapid onset and anticipated sublingual absorption kinetics, while 2–8-hour samples inform λz and t½ for both routes [1,3,4].

Scientific justification of sampling window

The schedule aligns with FDA and EMA recommendations to collect sufficient samples around tmax and across the elimination phase to enable precise estimation of Cmax, AUC, and λz, ensuring AUC extrapolation is minimized and terminal phase adequately characterized [1,3]. Labeling and clinical pharmacology sources indicate atropine exhibits rapid distribution with adult elimination half-life approximately 2–4 hours and clinically relevant unchanged renal excretion, supporting an 8-hour window for robust PK characterization after single 0.5–1.0 mg doses by sublingual and IV routes [2,4]. Interpretive synthesis: The design balances precision in early absorption with subject burden while meeting guidance-consistent criteria for rate and extent of exposure assessment [1,3].

Recording and verification of times; handling timing deviations

- Actual dosing time, all blood draw times, and urine collection start/stop times will be recorded using a 24-hour clock on source worksheets and transcribed to the eCRF. Timekeeping devices will be synchronized at least daily [1,5].
- The phlebotomist will record both planned nominal and actual times; any deviation from the schedule will be documented with reason. Per guidance, PK analyses will use actual times; nominal times may be used for tabular displays [1,3].
- Critical early timepoints (≤60 minutes) will be prioritized operationally to minimize deviation. Missed or substantially delayed samples will not be replaced; evaluability criteria will follow the SAP and guidance, with inclusion/exclusion decisions made under blinded review and documented [1,3,5].

Sample collection, processing, and handling

- Blood collection: Approximately 4 mL per draw into pre-chilled K2EDTA tubes; invert gently 8–10 times; maintain at 2–8°C and process within 30 minutes of collection [6,7].
- Centrifugation: 1500–2000 g for 10–15 minutes at 2–8°C; promptly transfer plasma into pre-labeled polypropylene cryovials using low-binding tips [6,7].
- Aliquoting: Prepare a minimum of two aliquots (primary and backup; target ≥0.3 mL each) per timepoint to mitigate freeze–thaw and analytical risk [6,7].
- Urine handling: Measure total volume for each interval; mix thoroughly; record pH; aliquot 2×2 mL into labeled cryovials [6,7].
- Labeling: Each vial labeled with protocol number, anonymized subject ID, period, treatment, matrix, nominal and actual collection time or interval, aliquot ID, and freezer location code. Barcoded labels will be used where available to support chain-of-custody [5].
- Storage: Freeze plasma and urine aliquots immediately at −70°C (or colder). Short-term refrigerated holds (≤24 hours at 2–8°C) are permitted only when pre-validated by the bioanalytical method; otherwise, freeze within 60 minutes. Avoid more than one freeze–thaw cycle before analysis unless dilution integrity is invoked [6–8].
- Shipment: Batch ship on dry ice to the central bioanalytical laboratory using validated, temperature-monitored containers. Shipments will include a packing list and chain-of-custody form; temperature excursions will be investigated and documented before sample release for analysis [5–8].
- Retention: Retain residual samples at −70°C (or colder) until database lock and completion of primary analyses, ISR, and any regulatory queries, then per sponsor records retention policy and applicable regulations [5–8].

Sample integrity, reconciliation, and chain-of-custody

- The site will maintain a sample accountability log linking each scheduled draw to collected aliquots, storage locations, shipments, and analysis status. Discrepancies will be reconciled promptly with root-cause documentation [5].
- Chain-of-custody will accompany all transfers, recording handover times, conditions, and personnel signatures. Temperature logs for freezers and shipments will be reviewed and filed; excursions will trigger impact assessment per SOPs and the bioanalytical stability file [5–8].

Bioanalytical methods and validation

- Analyte: Total atropine (racemate) quantified in human K2EDTA plasma as the primary matrix; urine quantified for exploratory Ae/fe. No enantiomer- or metabolite-specific analyses are planned for primary endpoints. Interpretive synthesis: Total atropine is the regulatory-relevant measurand for systemic exposure in absolute bioavailability assessments [1,3].

- Method: Validated LC–MS/MS with stable-isotope-labeled internal standard; calibration range anticipated to cover approximately 0.05–50 ng/mL for plasma with an LLOQ ≤20% of expected Cmax after sublingual 0.5–1.0 mg and IV 1.0 mg doses, based on published atropine assays in human plasma [6,9].
- Validation standards: The method will comply with FDA bioanalytical method validation guidance and EMA recommendations, including demonstration of selectivity, carryover, matrix effects, calibration model/weighting, accuracy (mean bias within ±15%; ±20% at LLOQ), precision (CV ≤15%; ≤20% at LLOQ), dilution integrity, recovery, and stability (bench-top, freeze–thaw, processed, long-term) in the study matrices [6,7]. Incurred sample reanalysis (ISR) will be performed on at least 10% of study samples spanning the concentration range, with predefined acceptance (repeat results within ±20% of original for the majority) [6,7].
- Stability: Long-term stability at −70°C (or colder) and short-term/processed stability will be established prior to analysis; acceptance requires measured concentrations within ±15% (±20% at LLOQ) of time-zero controls across validated durations. Any study-specific temperature excursion will be evaluated against the stability data to determine sample usability [6,7].
- Analytical oversight: The central bioanalytical laboratory will operate under GLP-like quality systems with method SOPs, instrument calibration/maintenance logs, audit trails, and documented analyst training. Analytical runs will include blanks, zero/STD calibrators, and duplicate QCs at LLOQ, low, mid, and high levels with run acceptance per guidance [6,7,9].

Pharmacokinetic analyses

- Parameters:

- For all treatments: Cmax, tmax, AUC0–t, AUC0–∞, λz, t½.
- For IV only: CL = Dose/AUC0–∞; Vd (apparent volume based on λz) = CL/λz.
- For sublingual only: CL/F and Vd/F derived analogously using AUC0–∞ and λz.
- Absolute bioavailability (F) of sublingual doses: F = (AUC0–∞,SL × DoseIV) / (AUC0–∞,IV × DoseSL). Dose-normalized AUC and Cmax will be summarized to support comparisons across doses [1,3].
- Exploratory renal endpoints: Ae0–8, fe0–8 = Ae0–8/Dose, and apparent renal clearance (CLR = Ae0–8/AUC0–8) [2–4].

- Methods: Noncompartmental analysis (NCA) using validated Phoenix WinNonlin (Certara) or equivalent validated software. λz will be estimated from ≥3 terminal points exhibiting log-linear decline; t½ = ln(2)/λz. AUCs will use the linear-up/log-down trapezoidal rule with extrapolation C last/λz [1,3].
- Analysis sets: PK concentration and parameter datasets will be derived per the SAP and guidance, using actual sampling times. Periods with critical early sampling failures, vomiting within the predefined window, or major dosing errors may be excluded from the per-protocol PK set after blinded review; all exclusions will be documented with rationale [1,3].
- Missing data and outliers: Missing concentrations will not be imputed. Outliers or anomalous profiles (e.g., aberrant Cmax due to sampling error) will be flagged via prespecified rules and evaluated under blinded review; sensitivity analyses will be performed when appropriate, and all decisions traceably documented in the PK analysis report and SAP [1,3].
- Reporting: The PK analyst/statistician designated by the sponsor will be responsible for NCA, dataset generation, and interpretive summaries. The bioanalytical laboratory will provide a validated concentration dataset, ISR results, and run acceptance documentation for auditability [5–7].

Deviations, sample loss, and analytical issues

- Any sample loss, hemolysis impacting analysis, thaw/refreeze excursion, or chromatographic failure will be recorded with impact assessment. Replacement sampling is not permitted post hoc. If the LLOQ is above observed concentrations at late times, λz/AUC0–∞ may be unestimable; AUC0–t will be used with explicit acknowledgment of extrapolation limits per guidance [1,3,6,7].

- Cross-contamination prevention measures include dedicated processing areas, change of gloves between samples, and verification of carryover on instruments with system blanks; any carryover event will trigger run rejection/reanalysis per SOPs [6,7,9].

Data capture and quality controls

- Source documents and eCRF entries will capture dosing, sampling, and processing timestamps; reconciliation checks will ensure internal consistency before database lock. Audit trails will document any corrections, aligned with ICH-GCP [5].
- Data transfers between bioanalytical and PK/statistical teams will use validated transfer specifications with checksum verification; discrepancies will be resolved under change control [5–7].

Transition to statistical methodology

Parameter derivations, data transformations, model specifications for central tendency and confidence intervals on exposure ratios (low-dose sublingual vs IV; high-dose sublingual vs IV; low vs high sublingual), handling of tmax (nonparametric), and criteria for inclusion in per-protocol and safety populations will follow the Statistical Analysis Plan in accordance with FDA and EMA guidance for bioavailability assessments [1,3].

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8.2 Safety Assessments

8.2.1 Physical Examination

Timing, scope, and purpose

Physical examinations will be performed to confirm eligibility, establish baseline health status, and provide ongoing safety surveillance given atropine's antimuscarinic effects (e.g., tachycardia, mydriasis, xerostomia, urinary retention) [1,4,5]. The schedule and content are specified to meet Good Clinical Practice expectations for protocol-defined assessments and auditable documentation [1,2].

Timepoint	Type and scope	Measurements captured	Purpose
Screening (Day −28 to −1)	Complete physical examination of all major systems	Height, weight, BMI; general exam; HEENT; cardiovascular; respiratory; abdominal; musculoskeletal; neurological; skin	Determine eligibility and baseline status [1,2]
Each dosing day pre-dose (Days 1, 8, 15)	Focused physical examination targeted to systems relevant to atropine effects plus any unresolved prior findings	Weight, BMI (recalculated), targeted systems exam	Confirm continued eligibility and readiness for dosing; safety baseline [1,4,5]
Interim/unscheduled (anytime)	Focused or complete exam triggered by AEs, vital sign/ECG/lab abnormalities, or investigator concern	As clinically indicated	Evaluate and follow up clinically relevant findings; guide dose withholding/withdrawal [1]
End-of-Study (EOS) or Early Termination	Complete physical examination	Weight, BMI; all systems as at screening	Document return to baseline or residual abnormalities; close-out safety [1,2]

Interpretive synthesis: The above cadence provides a complete baseline and close-out assessment with targeted pre-dose and event-driven evaluations to detect and manage antimuscarinic adverse effects while minimizing burden in a healthy-volunteer Phase 1 crossover study.

Examination procedures and body systems assessed

- General appearance and vital context: overall habitus, distress, hydration, orientation, and speech; inspection for anticholinergic signs such as dry mucosae, flushed skin, and mydriasis when relevant [4,5].
- Head, eyes, ears, nose, throat (HEENT): pupils (size/reactivity to light), extraocular movements, conjunctivae/sclerae, oral cavity and mucosal moisture, dentition, oropharynx, thyroid/neck inspection and palpation [4–6].
- Cardiovascular: precordial inspection/palpation; heart rate and rhythm; presence of murmurs, rubs, gallops; peripheral pulses; dependent edema [4–6].
- Respiratory: inspection; respiratory effort; chest expansion; auscultation for breath sounds and adventitious sounds [6].
- Gastrointestinal: inspection; bowel sounds; palpation for tenderness, masses, organomegaly; assessment for decreased bowel sounds if anticholinergic effects suspected [4–6].
- Musculoskeletal: gait; range of motion; joint swelling/tenderness; muscle bulk and tone [6].
- Neurological: mental status; cranial nerves (including accommodation); motor strength; sensory function; reflexes; coordination; tremor or agitation [6].
- Integumentary: skin temperature, color, moisture, turgor, rashes, injection or venipuncture sites [6].
- Genitourinary (as indicated): suprapubic fullness/tenderness if urinary retention suspected; focused exam as clinically warranted [4,5].
- Anthropometrics:

- Height measured once at screening using a stadiometer.
- Weight measured at screening, at each pre-dose assessment, and at EOS.
- Body mass index (BMI) calculated as weight (kg)/height (m)^2 at screening and recalculated at each dosing day and EOS using the most recent weight and screening height [7].

The complete system-by-system examination is required at screening and EOS; focused examinations at pre-dose and interim visits will prioritize systems pertinent to atropine safety (ocular, oral/ENT, cardiovascular, gastrointestinal, neurologic, and genitourinary) while addressing any previously noted abnormalities [1,4,5].

Personnel, conduct, and documentation

- Examinations will be performed by the principal investigator or qualified sub-investigators who are licensed healthcare professionals experienced in adult physical assessment, with delegation documented on the site delegation log per ICH-GCP [1].
- All findings will be contemporaneously documented in source records and transcribed to the electronic case report form (eCRF), including date/time, examiner identity, and system-level normal/abnormal results. Source-to-eCRF concordance will be maintained, and any corrections will be attributable, legible, contemporaneous, original, and accurate (ALCOA) in accordance with ICH-GCP and FDA recordkeeping requirements [1,8].
- Clinically significant abnormalities will be flagged in source and eCRF, assessed for relevance to eligibility and safety, and linked to any actions taken (e.g., dose delay/withhold, additional testing, referral) [1].

Evaluation and follow-up of clinically significant abnormalities

- Eligibility verification: At screening and each pre-dose assessment, abnormalities will be evaluated against protocol inclusion/exclusion criteria (e.g., glaucoma, tachyarrhythmias, gastrointestinal hypomotility, urinary retention) and atropine contraindications/precautions. Participants with findings that pose unacceptable risk or newly meet exclusionary thresholds (e.g., symptomatic tachycardia, sustained hypertension, ocular findings suggestive of angle-closure glaucoma) will not be dosed and may be withdrawn per protocol [1,4,5].
- Safety oversight: Any new or worsening abnormality temporally associated with study treatment will be evaluated for seriousness, severity, and causality, with targeted or complete re-examination performed as clinically indicated until resolution or stabilization. Event-driven focused exams will be conducted for anticholinergic manifestations, including:

- Cardiovascular (palpitations, tachyarrhythmias, blood pressure elevation) [4,5].
- Ocular (blurred vision, photophobia, fixed dilated pupils) [5].
- Gastrointestinal (abdominal pain, decreased bowel sounds/constipation) [4,5].
- Genitourinary (urinary hesitancy/retention) [4,5].
- Neurologic (confusion, agitation) [4,5].

- Repeat examinations: Clinically significant findings will be re-assessed at intervals appropriate to the condition (e.g., same day post-dose, next morning, or at follow-up) until they revert to baseline or a new stable baseline is established. Persistence at EOS will be documented with plans for follow-up or referral communicated to the participant as per site policies [1].
- Decision-making and reporting: Examination findings informing dose interruption, withdrawal, or expedited safety reporting will be documented with medical rationale, consistent with ICH-GCP responsibilities and FDA safety/recordkeeping expectations [1,8].

Alignment with other safety assessments

Physical examination findings will be integrated with vital signs, ECGs, and clinical laboratory results to support real-time safety decisions and comprehensive adverse event characterization, consistent with protocol-defined safety monitoring for healthy volunteer bioavailability studies [1,3]. Interpretive synthesis: Given atropine's rapid onset and short half-life, focused post-dose examinations will be

prioritized during the first 4 hours after dosing alongside vital signs monitoring to identify early antimuscarinic effects that may warrant intervention [4,5].

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8.2.2 Vital Signs and ECGs

Overview

Vital signs and electrocardiograms (ECGs) will be collected to monitor acute antimuscarinic effects of atropine, including tachycardia and potential conduction abnormalities, and to ensure participant safety in this healthy-volunteer crossover study [5,6]. Procedures, timing, posture, and equipment will be standardized to minimize variability and support auditable, GCP-compliant records [1,4].

Schedule of assessments

Study day/time	Blood pressure (BP) and heart rate (HR)	Respiratory rate (RR)	Body temperature (Temp)	12-lead ECG
Screening (Day −28 to −1)	1 set after ≥5 min seated rest	1 measurement (60 s)	1 measurement (oral)	1 standard 12-lead
Each dosing day pre-dose (within 60 min before dosing)	2 consecutive sets after ≥5–10 min seated rest; average recorded (repeat if difference in SBP >5 mmHg) [1]	1 measurement (60 s)	1 measurement (oral)	Triplicate ECGs separated by ≥1 min after ≥10 min supine rest; QTcF calculated [2,3]
Post-dose 0–60 min	At 5, 10, 15, 20, 30, 45, 60 min (seated) [interpretive synthesis to capture early atropine effects]	30 and 60 min	60 min	Triplicate at 30 min after ≥10 min supine rest [2,3]
Post-dose 60–240 min	90, 120, 180, 240 min (seated)	120, 240 min	240 min	Single ECGs at 120 and 240 min (supine, after ≥5–10 min rest)

Unscheduled (as indicated)	As clinically indicated for symptoms/abnormalities	As indicated	As indicated	Repeat immediate single or triplicate ECGs for new/worsening abnormalities

Interpretive synthesis: The schedule emphasizes dense early post-dose BP/HR monitoring to detect peak antimuscarinic effects while incorporating predose and time-matched ECGs around expected peak concentrations and at later times to surveil intervals and rhythm, consistent with ICH E14 principles for QT/QTc oversight outside a dedicated TQT study [2].

Measurement procedures and standardization

- General conditions:

- Abstain from food and drink per protocol restrictions; avoid talking, active movement, and phone use during measurements. Use quiet, temperature-controlled rooms. Timekeeping devices will be synchronized daily.
- The same arm, cuff size, thermometer site, and ECG lead placement will be used within a participant across all timepoints when feasible [1,3].

- BP and HR:

- Participant seated with back supported, feet flat, legs uncrossed, arm supported at heart level; rest quietly ≥5 minutes before measurement; empty bladder beforehand when feasible [1].
- Use an appropriately sized cuff (bladder length 75–100% and width 37–50% of arm circumference) and a validated, calibrated automated oscillometric device; record SBP, DBP, HR, posture, arm, cuff size, and conditions [1].
- Pre-dose: obtain two measurements separated by ≥1 minute; average recorded unless discordant (SBP difference >5 mmHg or DBP >5 mmHg), in which case obtain a third and average the two closest values [1].
- Post-dose: single measurements at scheduled times; if abnormal, repeat after ≥5 minutes of rest and confirm per action algorithm below.

- RR:

- Measure unobtrusively over a full 60 seconds while the participant is at rest and not speaking; record posture and method (visual chest rise or device-derived if validated) [8].

- Temperature:

- Oral digital thermometer preferred; ensure no hot/cold liquids within 15 minutes; use the same site per subject across visits. Follow manufacturer instructions and site SOPs for cleaning and calibration [8].

- ECG posture and rest:

- Supine rest for ≥10 minutes before triplicate ECGs and ≥5–10 minutes before single follow-up ECGs; minimize muscle tension and talking; standard lead placement per AHA recommendations [3].

Equipment specifications, calibration, and quality controls

- BP devices: Use automated oscillometric monitors validated by an accepted protocol, maintained and calibrated per manufacturer schedule (at least annually) with documentation; cuffs of multiple sizes available; devices verified for correct time/date [1].
- Thermometers: NIST-traceable or manufacturer-certified calibration per site SOPs; single-use probe covers; disinfection per instructions [8].
- ECG systems: Digital 12-lead machines meeting professional standards for acquisition and measurement; paper speed 25 mm/s, amplitude 10 mm/mV, sampling rate typically ≥500 Hz, with automated interval measurements and digital storage of waveform files [3]. Device self-tests performed per manufacturer recommendations; calibration/maintenance logs retained.
- Time stamps and data integrity: All devices set to synchronized time daily. Source data and eCRF entries will be attributable, legible, contemporaneous, original, and accurate per ICH-GCP; audit trails will capture any corrections [4].

ECG acquisition, intervals, and interpretation

- Type: Standard resting 12-lead ECGs.
- Replicates: Triplicate ECGs (separated by ≥1 minute) at pre-dose and 30 minutes post-dose to reduce measurement variability for QT/QTc; single ECGs at 120 and 240 minutes [2,3].
- Measurements:

- Automated measurements for PR, QRS, QT, RR, and QTc; QT corrected using Fridericia's formula (QTcF = QT/RR^1/3) given better performance than Bazett at variable heart rates [2].
- For each triplicate set, the mean of the replicates will be used for analysis and safety review.

- Review process: All ECGs will be reviewed by a qualified physician investigator; automated intervals will be confirmed when abnormal or borderline. Cardiology consultation will be sought for clinically significant abnormalities or persistent changes [2,3].

Criteria for abnormal or clinically significant findings and actions

- BP/HR thresholds and actions (interpretive synthesis informed by guideline thresholds and atropine pharmacology) [1,5–7]:

- Hypertensive urgency/crisis: SBP ≥180 mmHg or DBP ≥120 mmHg confirmed on repeat after ≥5 minutes rest → immediate clinical assessment; additional measurements every 5–10 minutes; consider withholding further study procedures, initiate medical management per site policy, and consult physician; hospitalization if indicated [7].
- Symptomatic hypotension or SBP <90 mmHg with symptoms (e.g., presyncope) → place supine, repeat BP/HR, assess; manage clinically; withhold further procedures; evaluate for withdrawal if persistent.
- Tachycardia: sustained HR ≥130 bpm or any symptomatic tachyarrhythmia → repeat vitals, obtain immediate ECG, clinical evaluation; consider IV fluids and other supportive measures; withhold/terminate study procedures; evaluate for withdrawal if persistent [5,6].

- ECG thresholds and actions per ICH E14 principles [2]:

- QTcF >500 ms or increase from pre-dose baseline >60 ms (confirmed on immediate repeat ECGs) → treat as clinically significant; urgent medical evaluation; continuous monitoring if indicated; withhold further study procedures and consider permanent discontinuation from study.
- New second- or third-degree AV block, new atrial fibrillation/flutter, sustained ventricular arrhythmia, or marked ST-T changes suggestive of ischemia → immediate clinical evaluation, repeat ECGs, consider emergency care; withdraw from dosing and from study as clinically indicated.
- Borderline QTcF values (men >450–500 ms; women >470–500 ms) or increase >30–60 ms: repeat ECGs (triplicate) within 15–30 minutes; enhanced monitoring; dosing in subsequent periods may be withheld pending medical review [2].

- Repeat procedures:

- Any abnormal vital sign will be rechecked after ≥5 minutes rest; ECGs will be repeated immediately (single or triplicate per above) when machine quality flags, excessive artifact, or abnormalities are detected.
- Persistent abnormalities will prompt targeted safety assessments (labs, physical exam) and adverse event documentation; criteria for discontinuation or withdrawal will be applied per protocol and medical judgment.

Data capture, storage, and traceability

- Source documentation will include date/time, posture, rest duration, device ID, arm/cuff size, measurement values, and staff initials for each vital sign; ECG printouts/digital files will include participant ID, date/time, lead calibration, paper speed, and device ID [1,4].
- Digital ECG waveform files and automated measurements will be archived on secure servers with role-based access, backed up per site SOPs; de-identified filenames will link to the eCRF. Paper artifacts (if any) will be scanned and filed with the investigator site file.

- All abnormal results, repeats, clinical reviews, and actions will be documented contemporaneously, with linkage to adverse event records where applicable, in compliance with ICH-GCP [4].

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8.2.3 Clinical Laboratory Assessments

Scope and overview

Clinical laboratory evaluations will be performed to confirm eligibility, establish baselines, and monitor safety in healthy adult participants receiving single-dose atropine by sublingual or intravenous routes. Assessments include hematology, clinical chemistry (with renal function by eGFR), urinalysis, infectious disease screening, pregnancy testing, and a urine drugs-of-abuse screen at screening. All laboratory services will be performed by a College of American Pathologists (CAP)–accredited and Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory in the United States; CAP accreditation and CLIA certification ensure analytical quality systems, participation in proficiency testing, and use of validated methods with established reference intervals [1–3]. Laboratory data will be managed and transferred under Good Clinical Practice (GCP) with attributable, contemporaneous documentation and auditable data flows; electronic records and signatures will comply with applicable U.S. regulations for electronic records where used [1,4].

Schedule and timepoints

Timepoint	Hematology (CBC with differential and platelets)	Chemistry (renal, hepatic, electrolytes, glucose, albumin/TP, eGFR)	Urinalysis	Infectious disease screening	Pregnancy testing (females of childbearing potential)	Urine drugs of abuse
Screening (Day −28 to −1)	Yes	Yes (includes TSH)	Yes	HIV-1/2 Ag/Ab; HBV (HBsAg, anti-HBs, total anti-HBc); HCV Ab with reflex NAT if Ab+ [5,6]	Serum hCG	Yes
Pre-dose each period (Days 1, 8, 15; within 24 h before dosing)	Yes	Yes	Yes	No (unless required to confirm status)	Urine hCG (same day, prior to dosing)	No
Post-dose (same day; ~8 h)	No (unless clinically indicated)	No (unless clinically indicated)	No (unless clinically indicated)	No	No	No
End-of-Study (EOS) or Early Termination (within 2–7 days after last dose)	Yes	Yes	Yes	No (unless new risk identified)	Serum or urine hCG	No

Interpretive synthesis: Predose assessments each period confirm continued eligibility and baseline comparability; EOS testing documents return to baseline or identifies residual abnormalities in accordance with GCP oversight for healthy volunteer studies [1].

Parameters and analytes

- Hematology:

- Complete blood count with differential and platelets: hemoglobin, hematocrit, red blood cell indices (MCV, MCH, MCHC, RDW), total and differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils), and platelet count [1].

- Clinical chemistry:

- Renal: blood urea nitrogen (BUN), creatinine, estimated glomerular filtration rate (eGFR) reported using a current CKD-EPI race-free equation; serum electrolytes (sodium, potassium, chloride, bicarbonate/CO2) [7].
- Hepatic: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (with direct fraction if elevated), total protein, albumin.
- Metabolic: glucose; calcium, magnesium, phosphorus.
- Thyroid: thyroid-stimulating hormone (TSH) at screening to support exclusion of thyroid disease.

- Urinalysis:

- Macroscopic and dipstick: color, appearance, specific gravity, pH, leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, occult blood.
- Microscopy (performed reflexively per laboratory algorithm when dipstick positive or as clinically indicated): red blood cells, white blood cells, epithelial cells, casts, crystals, bacteria/yeast [8].

- Infectious disease screening (screening only):

- HIV-1/2 antigen/antibody combination immunoassay with supplemental testing per CDC diagnostic algorithm [5].
- Hepatitis B: HBsAg, total anti-HBc, and anti-HBs to identify current or past infection and immunity per CDC recommendations [6].
- Hepatitis C: anti-HCV with reflex nucleic acid test (NAT) for HCV RNA when antibody reactive [6].

- Pregnancy testing:

- Serum hCG at screening; urine hCG on each dosing day prior to dosing; serum or urine hCG at EOS for early termination confirmation as appropriate [1].

- Urine drugs of abuse (screening only):

- Multi-panel immunoassay per laboratory standard to support exclusion of recent illicit drug use; positives may be confirmed by a specific method per laboratory SOPs [1].

Participant preparation and fasting

- Screening: Participants will be instructed to present after an overnight fast (approximately 8 hours) when feasible to standardize glucose interpretation; water is permitted. If fasting is not feasible, results will be interpreted using laboratory-provided reference intervals and clinical context [1].
- Dosing days: Predose safety laboratories will be drawn prior to dosing under the study's dietary restrictions (no food or drink for 1 hour pre-dose); additional fasting is not required beyond protocol-specified restrictions.

Sample collection, labeling, processing, handling, and storage

- General:

- All specimens will be collected using standard phlebotomy and clean-catch urine procedures per site SOPs. Tubes and containers will be labeled at collection with protocol number, anonymized subject ID, visit/timepoint, date/time of collection, and specimen type; barcodes will be used where available to support positive identification and chain-of-custody [1].

- Hematology:

- Collect whole blood into EDTA tubes; invert gently per manufacturer instructions; store at ambient temperature and analyze within laboratory-validated stability windows.

- Chemistry and serology (including HIV/HBV/HCV, TSH, serum hCG):

- Collect into serum separator tubes; allow clotting (per tube instructions), centrifuge, and separate serum. Refrigerate at 2–8°C for short-term holds; if shipment is required, maintain cold chain per laboratory instructions. Aliquots for reflex testing may be stored frozen at ≤−20°C per laboratory stability data.

- Urinalysis and urine hCG:

- Midstream clean-catch urine in sterile containers; analyze promptly per laboratory SOP. If delayed, refrigerate at 2–8°C and analyze within validated windows to avoid degradation of labile analytes [8].

- Shipment:

- When off-site analysis is used, specimens will be packaged with appropriate secondary containment and cold packs or dry ice as applicable, using temperature-controlled, monitored logistics. A packing list and chain-of-custody documentation will accompany each shipment; temperature excursions will be documented and assessed before analysis release [1].

- Retention:

- Residual specimens may be retained by the laboratory for quality assurance or reflex testing within validated stability; otherwise, disposal will follow laboratory policy and applicable regulations [1].

Laboratories, accreditation, and compliance

- Clinical safety and screening laboratories will be performed by laboratories holding current CLIA certification and CAP accreditation. CLIA establishes quality standards for laboratory testing in the U.S., including personnel qualifications, quality control, proficiency testing, and result reporting; CAP accreditation provides comprehensive peer-reviewed assessment of laboratory quality systems [2,3].
- Laboratory methods will be those validated and routinely used by the performing laboratory; reference intervals and units will be the laboratory's current standards for adult populations [2,3].

Data handling, ranges/units, reporting, and transfer

- Each laboratory result will be reported with the performing laboratory's units and reference intervals. Out-of-range values will be flagged by the laboratory; the site will verify clinical significance in context. All laboratory reports (including supplemental test algorithms for HIV/HBV/HCV) will be retained in source and transcribed to the eCRF under GCP ALCOA principles; electronic data transfers will use validated specifications with audit trails. Where electronic systems are used for acquisition or signature, compliance with applicable electronic records regulations will be ensured [1,4].
- For HIV diagnosis, HBV and HCV screening, results will be interpreted per CDC algorithms; reactive or indeterminate results will be managed with the recommended supplemental tests (e.g., HIV supplemental antibody differentiation, HCV RNA) [5,6].

Review of abnormalities, repeat testing, clinical follow-up, and documentation

- The investigator (or qualified sub-investigator) is responsible for medical review and interpretation of all laboratory results, integration with clinical findings, and determination of clinical significance per GCP [1].
- Abnormal results will be:

- Verified for pre-analytical or analytical errors (e.g., hemolysis, specimen mix-up) and repeated promptly when appropriate, especially when results impact eligibility, dosing, or safety.
- Followed clinically until resolution or stabilization. Persistent or clinically significant abnormalities will be evaluated with targeted history/exam and, if indicated, additional tests or referral. New findings meeting exclusion criteria prior to a dosing day will preclude dosing in that period; post-dose clinically significant abnormalities will be reported as adverse events.
- Managed according to condition severity; infectious disease positives will be disclosed and confirmed per CDC algorithms with counseling/referral per site policy [5,6].

- For renal function, eGFR will be used alongside serum creatinine to assess kidney status; if eGFR is unexpectedly low, confirmatory repeat testing will be considered, recognizing the updated race-free CKD-EPI equation improves accuracy and equity in reporting [7].
- All actions (repeats, clinical decisions, referrals) will be documented in source and eCRF with date/time, rationale, and outcome, maintaining traceability.

Quality controls and traceability

- The site will maintain logs for specimen collection, processing, storage, and shipment with chain-of-custody documentation. Device calibration and maintenance supporting specimen processing (e.g., centrifuges, refrigerators) will be recorded per SOPs. Deviations (e.g., temperature excursions, delayed processing) will be assessed for impact and documented, with mitigation as needed [1].
- The sponsor will ensure that only laboratories meeting CLIA/CAP requirements are engaged and that laboratory certifications are current throughout the study [2,3].

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8.2.4 Adverse Events and Serious Adverse Events

Definitions and scope

- Adverse event (AE): any untoward medical occurrence in a participant administered a study intervention that does not necessarily have a causal relationship with the intervention, including any unfavorable and unintended sign, symptom, or disease temporally associated with its use [1].
- Serious adverse event (SAE): an AE that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or is an important medical event that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed [2].
- Severity (intensity) is distinct from seriousness and will be graded using NCI CTCAE v5.0 where a specific term exists; otherwise, severity will be categorized as mild (Grade 1), moderate (Grade 2), severe (Grade 3), life-threatening (Grade 4), or death (Grade 5) per CTCAE definitions [3].
- Causality: the investigator will assess the relationship of each AE to study intervention using the “reasonable possibility” standard from ICH and FDA; a structured approach (e.g., WHO-UMC categories: certain, probable/likely, possible, unlikely, conditional/unclassified, unassessable/unclassifiable) will be used to support consistency in judgment [2,4]. For regulatory reporting, an AE is considered “related” if there is a reasonable possibility that the drug caused the event [5].
- Expectedness: assessed against the Reference Safety Information (RSI), consisting of the U.S. Prescribing Information for atropine sulfate injection (NDA 209260) and atropine sulfate ophthalmic solution 1% (NDA 206289). Antimuscarinic effects such as tachycardia, palpitations, mydriasis, blurred vision, photophobia, dry mouth (xerostomia), decreased sweating, urinary retention, constipation, flushing, and CNS effects (e.g., confusion, agitation at higher exposures) are considered expected for atropine [6,7].

Collection period, methods of elicitation, and documentation

- Collection window: All AEs will be collected from the time of first study intervention administration in Period 1 through the End-of-Study (EOS) visit or Early Termination. SAEs that occur after EOS and are judged by the investigator to be reasonably related to study intervention must be reported to the sponsor promptly upon awareness [1,5].
- Elicitation methods:

  - Open, non-leading questioning at prespecified timepoints (e.g., pre-dose and frequent post-dose contacts during the intensive monitoring window).
  - Participant self-report at any time during confinement and between visits.
  - Investigator/staff observation during procedures, vital signs/ECG assessments, and review of clinical laboratory results [1].

- Recording:

- Source documents will contemporaneously capture AE onset/stop dates and times, verbatim description, severity (CTCAE grade), seriousness, outcome, action taken with study intervention, relationship to study intervention, expectedness against RSI, and any therapies required. Concomitant medications/procedures used to treat AEs will be recorded [1–3,6,7].
- All AEs will be transcribed to the electronic case report form (eCRF) with audit trails; SAEs will additionally require a narrative summarizing chronology, pertinent medical history, relevant diagnostics, treatment, outcome, and causality/expectedness rationale [1].

Assessment and categorization

- Severity: graded per CTCAE v5.0; if a CTCAE term is not available, intensity will be mapped to the closest CTCAE grade using clinical descriptors (mild, moderate, severe, life-threatening) for consistency [3].
- Seriousness: determined per ICH E2A criteria; “life-threatening” refers to an event in which the participant was at immediate risk of death at the time of the event, not a hypothetical risk [2].
- Causality: assessed by the investigator at the time of AE detection and updated as new information emerges, using clinical judgment supported by temporal association, dechallenge/rechallenge (if applicable), alternative etiologies, and pharmacology; categorized per WHO-UMC and flagged as “related” or “not related” to align with the FDA reasonable-possibility standard for expedited reporting [2,4,5].
- Expectedness: determined by comparison with atropine RSI (U.S. Prescribing Information) for the dose and route context of this study; events not listed or of greater frequency/severity than described will be considered unexpected [5–7].

Reporting procedures and timelines

- Investigator to sponsor:

- SAEs: report immediately and no later than 24 hours of site awareness, irrespective of causality, using the sponsor SAE form; provide all available information and submit follow-up within 24–48 hours as additional details become available until resolution or stabilization [1,5].
- Non-serious AEs: record in source and eCRF within protocol-specified timelines; escalate to SAE reporting if seriousness criteria are subsequently met.

- Sponsor to regulators, investigators, and ethics committees:

- IND safety reports: the sponsor will report to FDA and all investigators any suspected unexpected serious adverse reaction (SUSAR) as soon as possible and no later than 15 calendar days after initial receipt of the information; fatal or life-threatening SUSARs will be reported as soon as possible but no later than 7 calendar days after initial receipt, with follow-up as necessary [5].
- The sponsor will also comply with ICH E2A standards for expedited reporting in applicable jurisdictions and ensure timely notification to all investigators and IRBs/IECs of new significant safety information [2,5].

- Investigator to IRB/IEC:

- Report SAEs and unanticipated problems per IRB/IEC policies and applicable regulations; maintain IRB/IEC continuing review and reporting obligations under 21 CFR 312.66 [8].

Coding and data standards

- All AEs will be coded to preferred terms and system organ classes using the Medical Dictionary for Regulatory Activities (MedDRA), employing the current version at database lock; coding changes made during medical review will be documented with rationale [9].

Special situations and protocol-specified considerations

- Pregnancy:

- Pregnancy in a participant or partner exposed via semen is not itself an AE but is a reportable special situation; the site must notify the sponsor within 24 hours of awareness. The pregnancy will be followed to outcome, with any associated complications meeting SAE criteria reported as SAEs. Study dosing is restricted to non-pregnant participants; inadvertent exposure will prompt immediate discontinuation and appropriate follow-up [1].

- Overdose, medication error, accidental exposure:

- Any overdose, incorrect route, dose misadministration, or accidental exposure will be documented and reported to the sponsor within 24 hours. If associated with an AE, assess seriousness and report as an SAE when criteria are met; otherwise, record as an AE of special interest [1,5].

- Protocol deviations and lack of efficacy:

- Protocol deviations are not AEs unless they result in a medical occurrence. As this is a healthy-volunteer bioavailability study without therapeutic intent, lack of efficacy is not applicable as an AE construct [1].

- Important medical events:

- Events that may not be immediately life-threatening or result in hospitalization but may jeopardize the participant or require intervention to prevent a serious outcome (e.g., marked tachyarrhythmia requiring urgent treatment, acute urinary retention requiring catheterization, severe anticholinergic syndrome) will be considered SAEs per ICH E2A [2,6,7].

On-study safety surveillance aligned to atropine pharmacology

- Given atropine's expected antimuscarinic effects, targeted surveillance for tachycardia, xerostomia, visual disturbances (mydriasis, blurred vision, photophobia), urinary retention, constipation, flushing, and CNS effects will be performed with dense early post-dose monitoring. These events are generally expected and typically transient at the study doses; however, clinically significant manifestations will be fully documented and managed, with expedited reporting if seriousness criteria are met [6,7]. This paragraph reflects interpretive synthesis of known atropine effects in healthy adults applied to the study's monitoring plan, with detailed case handling governed by the above standards.

Responsibilities, follow-up, and outcomes

- The investigator is responsible for initial detection, clinical evaluation, grading, causality/expectedness assessment, documentation, and timely reporting of AEs/SAEs; the sponsor's medical monitor will review safety data on an ongoing basis, request additional information, and determine reportability to authorities [1,5].
- All SAEs will be followed until resolution, return to baseline, stabilization, or a clinically acceptable endpoint. If an SAE leads to withdrawal, the outcome will be documented at EOS/early termination and updated if additional information becomes available [1].
- Concomitant medications and interventions used to treat AEs/SAEs will be recorded; rechallenge will not be performed solely for causality determination.

Cumulative safety review and data summarization

- Ongoing aggregate review: The sponsor will perform periodic cumulative reviews of blinded safety data, including AE line-listings and summary tables by treatment, severity, seriousness, relationship, and outcome, and will review any safety trends after each dosing period. Any emerging safety signal will be evaluated for clinical significance and regulatory reportability per ICH/FDA standards [1,2,5].
- Coding quality review: Medical review of coded terms will verify consistency with verbatim descriptions and CTCAE grading to support accurate aggregation and analysis [3,9].

- Final reporting: The clinical study report will include comprehensive summaries of treatment-emergent AEs/SAEs, relatedness, severity, discontinuations due to AEs, and narratives for all SAEs, in accordance with ICH E3/industry standards [1].

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8.3 Exploratory Assessments

Overview and purpose

Exploratory assessments will complement primary pharmacokinetic (PK) and prespecified safety evaluations to generate hypotheses about atropine exposure–response relationships, interindividual variability, and translatability of sublingual administration as a rapid countermeasure. These assessments include objective pharmacodynamic (PD) markers of antimuscarinic activity, additional biological matrices for exposure profiling, limited pharmacogenomic sampling for future exploratory analyses, and model-based PK/PD analyses. All exploratory assessments are hypothesis-generating, will not be used for confirmatory inference, and will be summarized descriptively or via exploratory modeling as appropriate [1,2].

Exploratory pharmacodynamic markers

- Quantitative salivary flow (unstimulated whole saliva; sialometry):
  - Rationale: Atropine reduces salivary secretion via muscarinic antagonism; objective sialometry provides a sensitive PD readout complementary to patient-reported xerostomia scores [3].
  - Methods: Unstimulated whole saliva collection using standardized timed passive drool into pre-weighed tubes (Navazesh method); flow rate (mL/min) determined gravimetrically (1 g ≈ 1 mL) under

controlled posture and conditions [3].
- Timepoints: Predose and approximately 10, 20, 30, 60 min, 2 h, and 4 h postdose to align with anticipated peak and declining antimuscarinic effects.

- Infrared pupillometry:

- Rationale: Systemic atropine produces mydriasis and reduced light reflex; automated pupillometry provides objective, repeatable measures of pupil size and reactivity as central/peripheral antimuscarinic PD markers [4].
- Methods: Automated infrared pupillometer under standardized ambient illumination and fixation; endpoints include resting pupil diameter (mm) and constriction velocity/percentage change to a standardized light stimulus [4].
- Timepoints: Predose and approximately 30, 60, 120, and 240 min postdose.

- Schirmer tear test (without anesthesia):

- Rationale: Muscarinic blockade reduces lacrimation; Schirmer testing quantifies aqueous tear production as an additional secretomotor PD marker [5].
- Methods: Standardized 5-minute Schirmer I strips placed in the lateral lower eyelid; wetting length (mm/5 min) recorded for each eye; lower value used for analysis [5].
- Timepoints: Predose and approximately 60 and 240 min postdose.

- Heart rate change as a PD effect:

- Rationale: Atropine increases heart rate via vagolytic effects; HR change from predose provides a sensitive systemic PD marker suitable for exposure–response analyses [6].
- Methods and source: HR derived from scheduled vital signs and ECGs per protocol; no additional procedures required [6].

Interpretive synthesis: The above PD panel triangulates antimuscarinic effects on exocrine secretion, ocular response, and autonomic tone to enable concentration–effect characterization across routes and doses in healthy adults.

Exploratory matrices for exposure profiling

- Saliva atropine concentrations:

- Rationale: Sublingual dosing may yield distinct oral cavity exposure profiles; saliva sampling enables assessment of early local/systemic kinetics and potential relationships to salivary flow inhibition [2].
- Matrix and timepoints: Whole saliva collected concomitantly with sialometry at predose, 10, 20, 30, 60 min, 2 h, and 4 h postdose.
- Analysis: Quantification by validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods adapted for saliva, meeting FDA bioanalytical method validation criteria (selectivity, accuracy, precision, calibration range, stability, matrix effects) [2].

- Urinary excretion (0–8 h pooled urine):

- Rationale: Atropine undergoes partial renal excretion unchanged; pooled urine permits exploratory estimation of fraction excreted (fe) and renal clearance to contextualize total clearance variability [7].
- Matrix and time window: Cumulative urine collected from 0 to 8 h postdose with total volume recorded; aliquots stored for analysis.
- Analysis: LC–MS/MS quantification of atropine; calculation of fe and renal clearance where feasible; methods validated per FDA guidance [2,7].

Pharmacometric analyses

- Exposure–response modeling:

- Concentration–HR modeling: Exploratory Emax or linear models relating plasma atropine concentrations to change in heart rate to characterize systemic vagolytic effects across routes/doses [6,8].
- Concentration–pupillometry/salivary flow: Exploratory models (e.g., inhibitory Emax for secretory endpoints) linking concentrations with changes in pupil metrics and salivary flow to quantify PD

potency and time course.
- Concentration–QTc modeling: If data permit, concentration–QTcF analyses will be explored to complement categorical ECG review, consistent with ICH E14 recommendations for concentration–QTc assessment outside a TQT study [1].

- Structural PK and PK/PD modeling:

- Beyond noncompartmental analysis, compartmental PK models (e.g., 1–2 compartment with first-order elimination; potential absorption transit for sublingual) may be fit to plasma (and saliva, if supportive) data to estimate absorption kinetics and interoccasion variability, and to support joint PK/PD modeling for the above endpoints. These analyses will follow principles in FDA exposure–response guidance and will be explicitly exploratory [8].

Model development, selection, and qualification will be performed using industry-standard software with goodness-of-fit diagnostics, visual predictive checks, and, where feasible, internal cross-validation; results will be summarized without inferential claims [8].

Pharmacogenomic and biomarker sampling

- Optional pharmacogenomic (PGx) sample banking:

- Purpose: To enable future hypothesis-generating analyses of genetic contributors to atropine PK/PD variability, including loci implicated in cholinergic signaling or drug metabolism/transport, recognizing current evidence linking specific genotypes to atropine disposition/response in healthy adults is limited [8].
- Matrix and handling: One EDTA whole-blood sample (~4–6 mL) collected once (e.g., predose in Period 1) for DNA extraction and storage under controlled conditions; any genotyping would be performed under validated laboratory procedures in accordance with applicable regulations and ethical approvals.
- Analyses: No prespecified genes are required for this protocol; any future exploratory analyses will be described in separate analysis plans and reported distinctly.

Sample collection, processing, and analytical methods

- Collection standards:

- Saliva and tear assessments will be performed under controlled ambient conditions minimizing recent oral intake per protocol restrictions; posture and timing standardized across visits.
- Urine pooled collection will record start/stop times and total volume; aliquots will be mixed and frozen promptly.
- Bioanalysis:

- All atropine quantitation in plasma, saliva, and urine will use validated LC–MS/MS methods meeting FDA bioanalytical method validation expectations, including calibration, QC acceptance criteria, carryover assessment, dilution integrity, reinjection reproducibility, and stability (bench-top, freeze–thaw, processed extract) [2].
- Laboratories:

- Exploratory bioanalyses will be conducted by a qualified bioanalytical laboratory using validated methods; PD device outputs (pupillometry) will be generated by calibrated equipment operated per manufacturer instructions and site SOPs [2,4].

Timing and schedule

Assessment	Matrix/device	Timepoints
Salivary flow rate (sialometry)	Whole saliva	Predose; 10, 20, 30, 60 min; 2, 4 h postdose
Saliva atropine concentration	Whole saliva	Predose; 10, 20, 30, 60 min; 2, 4 h postdose
Pupillometry	Infrared pupillometer	Predose; 30, 60, 120, 240 min postdose
Schirmer tear test	Schirmer strips	Predose; 60, 240 min postdose
Urinary excretion	0–8 h pooled urine	0–8 h postdose cumulative

Exploratory assessments will be performed in each dosing period unless precluded by safety or operational considerations; exact clock times relative to dosing will be recorded to enable time-aligned analyses.

Responsibilities, data handling, and reporting

- Performance and oversight:

- Exploratory PD procedures will be performed by trained site staff under investigator oversight; bioanalytical assays will be performed by a qualified laboratory with validated methods; pharmacometric analyses will be conducted by the sponsor or designated pharmacometricians.

- Data management:

- Exploratory data will be captured in source and eCRFs with synchronized timestamps to align with PK sampling. Device IDs, calibration records, and assay run/QC summaries will be maintained to support traceability [2].

- Reporting:

- Results will be summarized in separate exploratory listings, figures, and narratives, and, where applicable, integrated qualitatively with primary PK and safety findings. Model-based outputs will be clearly labeled as exploratory with no confirmatory inference or alpha-controlled hypothesis testing [1,8].

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9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

Primary statistical hypotheses and decision framework

The primary inferential framework follows established bioequivalence methodology applied to

single-dose pharmacokinetic (PK) comparisons in a 3-period crossover, using logarithmic transformation and linear mixed-effects modeling of PK parameters, with conclusions based on two one-sided tests (TOST) equivalent to two-sided 90% confidence intervals (CIs) for geometric mean ratios (GMRs) [1–3]. The overall Type I error rate is controlled at α=0.05 via TOST, consistent with regulatory guidance for equivalence assessment [1–3].

For each prespecified pairwise comparison—low-dose sublingual (SL) vs IV, high-dose SL vs IV, and low- vs high-dose SL—the null hypothesis (H0) is non-equivalence and the alternative hypothesis (H1) is equivalence for the formally tested PK parameters, evaluated against predefined acceptance limits:

- H0: The true GMR lies outside 80.00%–125.00%.
- H1: The true GMR lies within 80.00%–125.00%.

Consistent with regulatory principles, equivalence will be concluded for a parameter and comparison when the two-sided 90% CI for the GMR of log-transformed values lies entirely within 80.00%–125.00% [1,2]. For comparisons involving different nominal doses (e.g., 0.5 mg SL vs 1.0 mg IV), analyses will use dose-normalized PK parameters to support valid GMR interpretation; in parallel, absolute bioavailability (F) will be estimated as a descriptive parameter using dose-adjusted AUC ratios with 90% CIs, recognizing that equivalence to the IV route is not a regulatory requirement for absolute bioavailability estimation [2,3]. This dual approach aligns with guidance that extent of exposure is characterized primarily by AUC0–t, with AUC0–∞ supportive when the terminal phase is well characterized [1–3].

Parameters subject to formal hypothesis testing

- Formally tested (on the log scale): Cmax and AUC0–t for each pairwise comparison noted above; AUC0–∞ will be analyzed as supportive and tested analogously when reliable extrapolation (<20% of AUC extrapolated) is obtained, consistent with regulatory expectations for extent of exposure [1–3].
- Not tested for equivalence (descriptive/nonparametric): tmax (summarized by median and nonparametric 90% CI for treatment differences), t½, CL/F, and Vd/F, as per guidance that tmax is typically analyzed nonparametrically without equivalence criteria and that derived disposition parameters are descriptive in single-dose crossover settings [1,2].

Unless otherwise noted, PK parameters will be log_e-transformed prior to analysis; no untransformed analyses are planned for Cmax or AUC. tmax will be analyzed untransformed using nonparametric methods [1,2].

Statistical model and estimation

For log-transformed Cmax, AUC0–t, and (when applicable) AUC0–∞, a linear mixed-effects model will be fitted with fixed effects for sequence, period, and treatment, and a random effect for subject nested within sequence, in accordance with standard bioequivalence methodology for crossover designs [1,2]. From the model, least-squares means will be estimated for each treatment and used to compute GMRs and their two-sided 90% CIs on the original scale by exponentiation. Equivalence limits of 80.00%–125.00% will be applied uniformly to tested parameters [1,2]. This modeling approach, transformation, and CI-based decision rule conform to ICH E9 principles and FDA/EMA bioequivalence guidance [1–4].

For absolute bioavailability, F will be estimated descriptively as F = (AUC_SL/Dose_SL)/(AUC_IV/Dose_IV), with 90% CIs derived from the mixed-effects framework using dose-normalized AUCs; these estimates will be presented irrespective of equivalence conclusions, consistent with FDA guidance for BA studies in NDAs/INDs [3].

Alignment of alpha level and confidence intervals

TOST at the 5% level corresponds to two-sided 90% CIs for the GMRs; this CI level and the 80.00%–

125.00% limits are the customary regulatory standards for systemic small-molecule bioequivalence and are adopted here for the formal PK comparisons, while acknowledging that the overarching clinical objective is to characterize absolute and relative bioavailability of sublingual atropine to the IV reference [1–3].

Secondary and exploratory analyses

- Secondary safety and tolerability endpoints (including treatment-emergent AEs and xerostomia scores) will be summarized descriptively without formal equivalence testing or alpha allocation, consistent with ICH E9 recommendations regarding confirmatory versus exploratory objectives [4].
- Exploratory analyses (e.g., additional PK/PD relationships) will be hypothesis-generating without inferential claims; any CIs presented will be interpreted descriptively without formal decision thresholds [4].

Analysis populations and coherence with design

The primary equivalence analyses will be conducted in the PK-evaluable (per-protocol) population from the randomized three-period crossover, with supportive descriptive summaries in the safety population. Sequence, period, and treatment effects will be modeled as specified above, ensuring coherence with the crossover design and regulatory expectations for BE/BA analyses [1–4].

References

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9.2 Analysis Sets

Overview

Analysis populations for statistical evaluation will be predefined and finalized before database lock, with membership determined by a sponsor-led Data Review Team (study statistician, clinical pharmacologist, and medical monitor) based on blinded review of protocol deviations, PK sample integrity, and bioanalytical quality control, consistent with ICH E9 principles and GCP documentation standards [1,6]. The primary analysis will use the Per-Protocol (PP) Set. Supportive and sensitivity analyses will use the Full Analysis Set (FAS) and the Safety Set, as appropriate to the estimand and objective [1–4].

Full Analysis Set (FAS)

- Definition:

- All randomized participants who receive at least one dose of study intervention and have at least one post-dose, quantifiable plasma atropine concentration in any period. Period-level inclusion is permitted; a participant may contribute evaluable data for any period in which ≥1 quantifiable post-dose concentration is available [1–4].

- Use:

- Supportive analyses of primary PK parameters (descriptive and model-based where feasible) and secondary/exploratory PK/PD summaries. No imputation of missing PK concentrations is planned; PK parameters will be derived from observed data only [1–4].

- Handling of incomplete periods:

- For pairwise comparisons, a participant-period contributes only when both treatments required for that contrast are evaluable in the same participant. Mixed-effects models inherently accommodate the resulting imbalance without imputation [1–3].

Per-Protocol (PP) Set (Primary)

- Definition:

- A subset of the FAS including participant-periods meeting all of the following:
  - Correct randomized treatment received per assigned sequence and route, at the protocol-specified nominal dose and within allowable dosing-time windows [2–4].
  - Washout between consecutive periods approximately 7 days; deviations are major if judged to risk carryover based on atropine half-life and PK profile [2–4].
  - PK sampling adequate to estimate AUC0–t and Cmax with reliable characterization of the terminal phase when AUC0–∞ is derived:
    - Cmax captured (no missing samples in the critical window spanning nominal 10–60 min postdose unless interpolation still identifies a plausible peak).
    - At least three quantifiable concentrations in the terminal log-linear phase for estimation of λz and t½ when AUC0–∞ is derived, with percent extrapolated AUC ≤20% for AUC0–∞ to be considered reliable [2–4].
    - If AUC0–∞ is unreliable (>20% extrapolated) but AUC0–t is robust, the period may remain in PP for AUC0–t/Cmax analyses and be excluded for AUC0–∞ analyses only [2,3].
  - Bioanalytical runs meet predefined acceptance criteria (calibration/QC within FDA validation limits); sample integrity not compromised (e.g., no documented temperature excursion invalidating results) [5].
  - No major protocol deviations with potential to meaningfully alter PK (see Major Protocol Deviations below).

- Use:

- Primary analyses for Cmax and AUC0–t (and AUC0–∞ when reliable) using the specified mixed-effects model for the randomized three-period crossover [2–4].

- Period-wise eligibility and contrasts:

- For each pairwise comparison, a participant contributes only the participant-periods containing both treatments of interest that meet PP criteria. Participants missing one period may still contribute to contrasts supported by their remaining valid periods [2–4].

Safety Set

- Definition:

- All participants who receive any amount of study intervention in any period, analyzed as-treated by period. Safety summaries will be based on treatment received in each dosing period, with treatment-emergent events accruing from dosing through End-of-Study for that participant [1,6].

- Use:

- Summaries of adverse events, vitals/ECGs, clinical laboratories, and targeted tolerability measures (e.g., xerostomia), stratified by treatment and period.

Major protocol deviations affecting PK data

Major deviations are prespecified conditions that exclude a participant-period from the PP Set for PK analyses impacted by the deviation, with documentation and rationale retained per GCP. Examples include:

- Treatment administration:

- Wrong treatment, dose, or route; dosing out of sequence; dosing time outside allowable windows that could shift the concentration–time profile [2–4].
- Sublingual administration not per protocol (e.g., observed spitting/expulsion of a substantial portion of the dose; failure to maintain sublingual contact per instructions), when judged likely to alter absorption kinetics materially [2–4].

- Washout and carryover risk:

- Insufficient washout or protocol deviation introducing carryover risk based on atropine PK; such periods are excluded from PP for affected contrasts [2–4].

- Concomitant medication or prohibited intake:

- Use of prohibited medications or substances with known cholinergic/anticholinergic effects or PK interaction potential within restricted windows that could alter atropine PK/PD [2–4].

- Sampling and bioanalytical integrity:

- Missing critical PK samples precluding reliable estimation of Cmax or AUC0–t; inability to characterize terminal phase when AUC0–∞ is required; bioanalytical failure without successful repeat meeting validation criteria [2–5].

- Emesis/vomiting or expulsion related to dosing:

- Vomiting or expulsion judged to lead to loss of dose within a window likely to impact absorption (for immediate effects, generally within approximately 2× median tmax for the route), resulting in exclusion of the affected period from PP; inclusion may be retained for IV periods when systemic input is unaffected [3].

Decisions on classification and impact of deviations will be made by the Data Review Team using prespecified rules and clinical/pharmacokinetic judgment, prior to database lock, consistent with ICH E9 and EMA guidance on exclusions and outliers [1–3].

Handling of missing PK samples and profiles

- No imputation will be performed. Noncompartmental PK parameters will be derived from observed concentrations only [2–4].
- Inclusion rules:

- AUC0–t requires sufficient observed concentrations to the last quantifiable timepoint with appropriate trapezoidal integration; isolated missing samples are acceptable if they do not preclude identification of Cmax and reasonable delineation of the elimination phase contributing to AUC0–t [2–4].
- AUC0–∞ requires a reliably estimated terminal rate constant (≥3 quantifiable terminal points) and percent extrapolation ≤20%; otherwise, AUC0–∞ will be treated as unreliable and excluded from PP for that parameter while retaining AUC0–t/Cmax if adequate [2,3].

- Sensitivity to missingness:

- Sensitivity analyses will compare PP versus FAS results to assess robustness to missing PK data and period exclusions, aligned with ICH E9(R1) recommendations on sensitivity analysis to address deviations from ideal conditions [1].

Outliers and influential data

- No routine exclusion of statistical outliers is planned. If a data point is identified as an extreme or influential observation with a plausible underlying cause (e.g., documented dosing error, confirmed sample mix-up), the affected participant-period may be excluded from PP for impacted parameters with prospective rules and justification; primary results will be accompanied by supportive analyses including all data (FAS) and, if warranted, sensitivity excluding the identified outlier(s), in line with EMA/FDA recommendations [2,3].

Replacement subjects and evaluability

- Replacement:

- Participants who withdraw or are withdrawn before receiving any study intervention may be replaced to achieve approximately 12 PP-evaluable participants (about 4 per sequence). Replacement participants will receive new identifiers and be randomized per the allocation schedule [2–4].

- Partially dosed/partially assessed participants:

- Participants who receive any dose will be included in the Safety Set. Their evaluable periods may contribute to the FAS and, when criteria are met, to the PP Set for specific parameters and contrasts [1–4].

Finalization of analysis sets and documentation

- The Data Review Team will finalize the FAS, PP Set, and Safety Set prior to database lock and prior to any unblinded inferential analyses, with all decisions traceable in a contemporaneous data review memorandum and deviation listings, consistent with ICH E9 and GCP [1,6].
- All inclusions/exclusions will be listed by participant and period, with parameter-specific flags when necessary (e.g., PP for AUC0–t/Cmax but not for AUC0–∞) [2–4].

Sensitivity and supportive analyses across datasets

- Primary: PP Set for Cmax and AUC0–t (AUC0–∞ when reliable).
- Supportive:

- FAS analyses to assess robustness to protocol deviations/missingness using the same mixed-effects model where convergence permits; descriptive summaries when modeling is not feasible [1–4].

- Safety Set for tolerability summaries and exposure–AE descriptions by treatment.

- Additional sensitivity:

- If exclusions due to potential carryover, administration errors, or identified influential observations materially affect conclusions, parallel analyses including and excluding the affected data will be presented and interpreted as sensitivity analyses (expert interpretation consistent with ICH E9(R1) and EMA BE guidance) [1–3].

References

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9.3 Statistical Methods

9.3.1 Pharmacokinetic Analyses

Overview and analysis approach

Pharmacokinetic (PK) parameters for atropine following single-dose administration of the sublingual test products (0.5 mg and 1.0 mg) and the intravenous (IV) reference (1.0 mg) will be derived from plasma concentration–time data using noncompartmental analysis (NCA) with actual sampling times. Trapezoidal integration will employ linear-up/log-down rules to improve estimation across rising and declining phases, consistent with regulatory expectations for BA/BE studies [1,2]. Parent atropine (active moiety) will be the primary analyte for all PK analyses; metabolite or enantiomer PK will not be used for primary interpretation, in line with guidance that bioavailability and equivalence assessments should generally be based on the parent drug unless a metabolite better reflects exposure [2].

Derived PK parameters and calculation methods

For each dosing period and participant with quantifiable data, the following parameters will be calculated:

- Cmax: Maximum observed plasma concentration.
- tmax: Time of first occurrence of Cmax (observed; no interpolation).
- AUC0–t: Area under the plasma concentration–time curve from time zero to the last quantifiable concentration, calculated via the linear-up/log-down trapezoidal method using actual times [1].
- λz: Apparent terminal elimination rate constant, estimated by log-linear regression of at least three terminal, descending, quantifiable concentrations selected algorithmically (highest adjusted fit quality and visual plausibility) [2].
- t½: Terminal half-life, t½ = ln(2)/λz [2].
- AUC0–∞: AUC0–t + Ct/λz, where Ct is the last quantifiable concentration; AUC0–∞ will be considered reliable when the extrapolated fraction (AUCextrap = 100 × (Ct/λz)/AUC0–∞) is ≤20% and the terminal phase is adequately characterized [1,2].
- For IV reference:

- CL: Total plasma clearance, CL = DoseIV/AUC0–∞ when reliable [1].
- Vz: Apparent volume of distribution during the terminal phase, Vz = CL/λz [1].

- For extravascular (sublingual) test products:

- CL/F: Apparent clearance, CL/F = DoseSL/AUC0–∞ when reliable [1].
- Vz/F: Apparent volume of distribution, Vz/F = (CL/F)/λz [1].

- Absolute bioavailability (descriptive): F = (AUC_SL/Dose_SL)/(AUC_IV/Dose_IV); 90% confidence intervals will be derived from the mixed-effects model using dose-normalized AUCs as described in Statistical Methods for PK Data [1].

Where AUC0–∞ is not reliable (e.g., >20% extrapolated or inadequate terminal phase), AUC0–t will be the extent-of-exposure parameter for comparison, consistent with EMA and FDA guidance [1,2]. Interpretive synthesis will be used to discuss concordance of AUC0–t and AUC0–∞ when both are available [1,2].

Evaluable PK profile criteria and terminal phase adequacy

A participant-period will be considered evaluable for parameter-specific analyses when:

- Sampling captures the absorption and distribution phases sufficiently to identify Cmax and delineate AUC0–t using actual times [1,2].
- For parameters requiring λz (t½, AUC0–∞, CL, Vz, CL/F, Vz/F): at least three terminal, descending, quantifiable concentrations are available within an apparent log-linear terminal phase, with an acceptable regression fit and AUCextrap ≤20% for AUC0–∞ to be considered reliable [1,2].
- No evidence of clinically meaningful carry-over is present (see Carry-over assessment) [2,3].

- Bioanalytical runs meet predefined acceptance criteria per validated methods (calibration/QC within limits), including assessment and control of carryover at the analytical level [4].

If AUC0–∞ is unreliable but AUC0–t and Cmax are robust, the period may be included for Cmax and AUC0–t while excluded from analyses relying on λz, consistent with guidance [1,2].

Handling of sampling deviations, missing data, and BLQ values

- Actual times: All PK calculations will use recorded actual sampling times rather than nominal times [1,2].
- Missing samples: No imputation will be performed. AUC0–t will be integrated over available quantifiable concentrations; isolated missing points are acceptable if they do not preclude identification of Cmax or reasonable delineation of the elimination phase contributing to AUC0–t [1,2].
- Below the lower limit of quantification (BLQ):

- Pre-dose BLQ values will be set to zero [2,4].
- Post-dose BLQ values prior to the first quantifiable concentration will be set to zero [2,4].
- Intervening BLQ values between quantifiable concentrations will be treated as missing (no substitution) and excluded from λz estimation [2].
- BLQ values occurring after the last quantifiable concentration will be ignored for AUC integration and excluded from λz estimation [2].

- Mistimed samples: When a critical timepoint is mistimed, the observed time will be used. If a cluster of critical early samples is missing or grossly mistimed such that Cmax cannot be reasonably identified, the period will be excluded for Cmax and AUC0–t in the Per-Protocol analysis as prespecified in Analysis Sets [2].

These conventions align with regulatory guidance emphasizing use of observed data and validated bioanalytical results without post hoc imputation, and with established practices for BLQ handling in BA/BE analyses [1,2,4].

Carry-over assessment and period exclusion rules

Carry-over will be assessed using predose (trough) concentrations in Periods 2 and 3:

- If the predose concentration exceeds 5% of that period's individual Cmax, the affected period will be excluded from primary PK analyses for that participant because of potential carry-over, per EMA and FDA recommendations [2,3].
- If predose is quantifiable but ≤5% of Cmax, the period may be retained, with sensitivity analyses performed as needed (expert interpretation) [2,3].
- Documented dosing/washout deviations judged to risk carry-over will trigger exclusion from the Per-Protocol Set for affected parameters, with supportive analyses in the Full Analysis Set as described in Analysis Sets [2].

Dose normalization and planned comparisons

For planned treatment comparisons, dose-normalized parameters will be used where appropriate to enable interpretable ratios across different nominal doses (e.g., 0.5 mg SL vs 1.0 mg IV), and absolute bioavailability will be estimated descriptively from dose-adjusted AUCs in accordance with FDA guidance for BA studies [1].

Software, validation, and responsibilities

- Software: PK computations will be performed using Phoenix WinNonlin (version 8.3 or higher) or equivalent validated NCA software. Calculations will use linear-up/log-down integration, log-linear regression for λz, and standard NCA algorithms consistent with regulatory principles [1,2].

- Validation and compliance: The analysis environment will be validated per sponsor SOPs and 21 CFR Part 11/annexed expectations for computerized systems used in clinical investigations, including access control, audit trails, and change control [5].
- Responsibilities: The sponsor's clinical pharmacology function (or a designated qualified vendor) will conduct PK data assembly, computation, quality review, and finalize PK parameter datasets for statistical analysis.

Data flow, verification, and data lock

- Data transfers: The bioanalytical laboratory will provide sample-level concentration data with run summaries, calibration, QC results, ISR outcomes, and stability statements under a validated method meeting FDA and ICH bioanalytical validation principles [4].
- Quality checks: The PK analyst will perform programmed checks for sequence/period consistency, sampling times, units, BLQ handling, terminal phase adequacy, predose concentrations, and algorithmic selection of λz points, with independent quality review documented prior to PK data lock.
- ISR and reanalysis: Incurred sample reanalysis (ISR) outcomes will be reviewed; if ISR acceptance criteria are not met or reruns replace original values per validated procedures, the final, accepted value will be used for PK computations, with all changes traceable [4].
- PK dataset lock: After resolution of data queries and finalization of evaluability flags (parameter-specific), a PK Analysis Dataset (ADaM-compliant where applicable) will be locked and transferred to biostatistics for modeling as specified in Statistical Methods for PK Data. Any post-lock changes will follow controlled change management and will be documented with re-lock if required [5].

Summaries and data presentation

- Continuous PK parameters (Cmax, AUC0–t, AUC0–∞, t½, CL/CL/F, Vz/Vz/F) will be summarized by treatment using descriptive statistics (n, mean, SD, CV%, median, min, max). tmax will be summarized by median and range; nonparametric summaries will be provided as appropriate [1,2].
- Individual and mean concentration–time profiles will be plotted by treatment using linear and semi-log scales. Goodness-of-fit diagnostics for λz selection will be archived.
- Parent only: No metabolite or enantiomer parameters are planned for primary summaries. Should exploratory metabolite or stereospecific analyses be performed, they will be summarized descriptively and clearly labeled as exploratory without inferential testing, consistent with guidance prioritizing parent drug for BA/BE characterization [2].

Linkage to PK assessments and statistical methods

These analyses align with the predefined PK sampling schedule and endpoints in Pharmacokinetic (PK) Assessments and will provide the parameter inputs for the mixed-effects comparisons described in Statistical Methods for PK Data, including calculation of geometric mean ratios and 90% confidence intervals for prespecified treatment contrasts [1,2].

References

1. U.S. Food and Drug Administration (FDA). Guidance for Industry: Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs—General Considerations. Silver Spring (MD): FDA; 2014. Available from: https://www.fda.gov/media/88254/download
2. European Medicines Agency (EMA). Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**). London: EMA; 2010. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf

3. U.S. Food and Drug Administration (FDA). Guidance for Industry: Statistical Approaches to Establishing Bioequivalence. Silver Spring (MD): FDA; 2001. Available from: https://www.fda.gov/media/70958/download
4. U.S. Food and Drug Administration (FDA). Guidance for Industry: Bioanalytical Method Validation. Silver Spring (MD): FDA; 2018. Available from: https://www.fda.gov/media/70858/download
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9.3.2 Statistical Methods for PK Data

Overview and analysis framework

Inferential analyses will follow established bioavailability/bioequivalence methodology using logarithmic transformation and linear mixed-effects modeling for primary PK parameters (Cmax, AUC0–t, and AUC0–∞ when reliable), with estimation of least-squares means (LSMeans), geometric mean ratios (GMRs), and two-sided 90% confidence intervals (CIs) based on two one-sided tests (TOST) at α=0.05 overall, consistent with FDA and EMA guidance and ICH statistical principles [1–4]. Although this is a descriptive absolute bioavailability study rather than a formal bioequivalence (BE) submission, GMRs and 90% CIs will be reported for prespecified pairwise comparisons: low-dose sublingual (SL) vs IV, high-dose SL vs IV, and low- vs high-dose SL. For interpretability across unequal nominal doses, analyses for SL vs IV will use dose-normalized AUCs; absolute bioavailability will be estimated as F = (AUC_SL/Dose_SL)/(AUC_IV/Dose_IV) with 90% CIs from the model applied to dose-normalized AUCs [2,3]. Where equivalence-type interpretation is informative, the conventional 80.00%–125.00% limits will be referenced; however, no regulatory BE conclusion is required for absolute bioavailability characterization in this setting [1–3].

Data transformations and analysis sets

- Log_e-transformation will be applied to Cmax, AUC0–t, and AUC0–∞ prior to modeling; no back-transformation correction is needed beyond exponentiation of LSMean differences to obtain GMRs and 90% CIs [1,2].
- tmax will be analyzed untransformed using nonparametric methods (see Nonparametric analyses) [2].
- Primary analyses will use the Per-Protocol (PP) Set, with supportive analyses in the Full Analysis Set (FAS), consistent with ICH E9 and prior section definitions; mixed-effects models inherently accommodate missing periods without imputation for evaluable contrasts [3,4].

Statistical model specification

- Model: Linear mixed-effects model fitted separately for each parameter (log_e Cmax, log_e AUC0–t, and log_e AUC0–∞ when reliable).
- Fixed effects: sequence, period, treatment.
- Random effects: subject nested within sequence (to capture between-subject heterogeneity) [1,2].
- Estimation: Restricted maximum likelihood (REML); denominator degrees of freedom via Satterthwaite or Kenward–Roger approximation as implemented in SAS; model convergence and diagnostics will be documented [1,4].
- From fitted models, LSMeans by treatment will be obtained; pairwise LSMean differences (test minus reference) and associated standard errors will be exponentiated to yield GMRs and two-sided 90% CIs on the original scale [1,2].

Equivalence margins and interpretive conventions

- For context and comparability to BE standards, the 80.00%–125.00% interval will be presented for Cmax and AUCs. Equivalence would be inferred if the 90% CI for the GMR lies fully within 80.00%–125.00% [1,2]. In this BA study, these intervals are descriptive and not used for a formal BE claim versus IV; dose-normalized AUCs support estimation of F and characterization of exposure [2,3].

Nonparametric analyses

- tmax will be summarized by treatment using medians and ranges. Between-treatment differences in tmax will be evaluated using a nonparametric approach appropriate for crossover data (e.g., Wilcoxon signed-rank on within-subject paired differences for the contributing periods) with Hodges–Lehmann point estimates and nonparametric 90% CIs; no equivalence margins are prespecified for tmax in accordance with guidance [1,2].

Variability estimation and reporting

- Intra-subject variability: Estimated from the residual mean square error (MSE) of the mixed model on the log scale and reported as within-subject CV% using CV_within = 100 × sqrt(exp(MSE) − 1) for each parameter and treatment/contrast as appropriate [1,2].
- Inter-subject variability: Estimated from the between-subject (subject-within-sequence) random effect variance and summarized as CV_between% using the analogous log-scale transformation [1,4].
- Variance components, standard errors, and 90% CIs for GMRs will be tabulated to support interpretation and potential future design planning [1–3].

Sensitivity, subset, and supportive analyses

- PP vs FAS: The primary PP analyses will be repeated in the FAS to assess robustness to protocol deviations and missing periods, per ICH E9(R1) recommendations on sensitivity analyses for deviations from ideal conditions [4].
- Parameter-specific evaluability: Where AUC0–∞ is unreliable (>20% extrapolated or inadequate terminal phase), supportive analyses will focus on AUC0–t and Cmax only, consistent with FDA/EMA guidance [2,3].
- Influential data: If prespecified rules identify influential observations attributable to documented causes (e.g., dosing error), sensitivity analyses including and excluding these data will be presented without changing the primary PP inference, aligned with EMA/FDA positions on outliers [1,2].

Handling of missing data, incomplete periods, and withdrawals

- No imputation of PK concentrations or parameters will be performed. Participants with one or more missing periods may still contribute to pairwise contrasts for which both treatments are evaluable in the same subject; the mixed model accommodates the resulting imbalance [1–3].
- Periods excluded from PP for a specific parameter (e.g., unreliable AUC0–∞) may still contribute to other parameters (e.g., AUC0–t, Cmax) when evaluable, as predefined in the Analysis Sets; all exclusions will be parameter-specific and documented prior to unblinding final analyses [2–4].

Highly variable drugs and design considerations

- No replicate or reference-scaled average bioequivalence procedures are planned because the study uses a three-period non-replicate crossover and aims to estimate absolute and relative bioavailability descriptively. Even if within-subject CV exceeds 30%, no widening of BE limits or two-stage designs will be applied; variability will be characterized and discussed descriptively in line with EMA and FDA guidance for non-replicate BA/BE studies [1–3].

Statistical software, validation, and documentation

- Software:

- Model-based analyses: SAS (version 9.4 or higher; SAS Institute Inc., Cary, NC, USA) PROC MIXED/GLIMMIX for mixed-effects models, with verification of convergence and d.f. method [1,4].
- PK computations: Phoenix WinNonlin (version 8.3 or higher; Certara) as specified in Pharmacokinetic Analyses, with linear-up/log-down integration [2,3].

- Validation and documentation:

- Programming will follow sponsor SOPs under 21 CFR Part 11 expectations for computerized systems with audit trails; outputs will be traceable to CDISC-compliant SDTM/ADaM datasets where applicable, consistent with FDA Study Data Technical Conformance Guide [5].
- Independent quality control (double programming or code review) will be performed for primary analyses and key listings/figures. Final statistical outputs will be reviewed and signed off by the lead biostatistician and the clinical pharmacologist/medical monitor per ICH E6(R2) [6].

Analysis populations, inclusion/exclusion for statistical datasets

- Primary dataset: PP Set as defined previously, restricted to participant-periods meeting dosing, washout, sampling adequacy, and bioanalytical integrity requirements; inclusion may differ by parameter (e.g., included for AUC0–t/Cmax but excluded for AUC0–∞) [2–4].
- Supportive dataset: FAS includes all dosed participants with at least one quantifiable post-dose concentration; used to replicate primary models where feasible and to provide descriptive statistics when modeling is not supported [3,4].
- Safety Set: All dosed participants, analyzed as treated by period; not used for inferential PK modeling.

Presentation of results

- Descriptive summaries: By treatment and sequence where relevant—n, mean, SD, CV%, median, min, max for log-scale back-transformed parameters; tmax by median and range [1–3].
- Inferential summaries: For each pairwise contrast, tables will present LSMeans (log scale), GMRs (original scale), two-sided 90% CIs, within-subject CV%, and model diagnostics.
- Graphical outputs: Individual and mean concentration–time profiles (linear and semi-log), individual spaghetti plots, and forest plots of GMRs with 90% CIs by parameter. All figures and tables will reference the analysis population and parameter-specific evaluability.
- Listings: Participant-level PK parameters, evaluability flags, protocol deviations affecting PK, and predose concentrations indicating potential carryover will be listed.

Decision rules and multiplicity

- No alpha allocation across multiple endpoints is required beyond TOST for each contrast/parameter because formal BE claims are not sought; 90% CIs are presented

descriptively in alignment with regulatory conventions for BA/BE characterization [1–3]. Interpretive statements will clearly distinguish descriptive estimation from confirmatory BE conclusions (expert interpretation).

Traceability and audit readiness

- All modeling specifications, dataset versions, and output generation steps will be archived with version control. The statistical section of the clinical study report will reproduce primary and supportive results, with full traceability to the SAP and the locked ADaM datasets, consistent with ICH E9 and E6(R2) [4,6] and FDA data standards expectations [5].

References

1. U.S. Food and Drug Administration (FDA). Guidance for Industry: Statistical Approaches to Establishing Bioequivalence. Silver Spring (MD): FDA; 2001. Available from: https://www.fda.gov/media/70958/download
2. European Medicines Agency (EMA). Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**). London: EMA; 2010. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf
3. U.S. Food and Drug Administration (FDA). Guidance for Industry: Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs—General Considerations. Silver Spring (MD): FDA; 2014. Available from: https://www.fda.gov/media/88254/download
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6. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). E6(R2) Guideline for Good Clinical Practice. Geneva: ICH; 2016. Available from: https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf

9.4 Sample Size Determination

Overview and regulatory framework

The planned sample size is grounded in established bioavailability/bioequivalence (BA/BE) principles using two one-sided tests (TOST) on log-transformed pharmacokinetic (PK) parameters, with Type I error α=0.05 (equivalently, two-sided 90% confidence intervals) and within-subject variability (intra-subject coefficient of variation, CVw%) as the key determinant in a three-period crossover design, consistent with FDA and EMA guidance [1–3]. Given the descriptive absolute bioavailability (F) objective but inferential estimation of geometric mean ratios (GMRs) and 90% CIs for Cmax and AUCs, planning follows conventional BE-style power calculations to ensure adequate precision and operating characteristics; alignment with the crossover design and analytical model is described in the Scientific Rationale for Study Design and Statistical Methods sections [1–3].

Variability assumptions and rationale

- Key parameters: Cmax and AUC0–t (AUC0–∞ when reliable) are the parameters driving sample size because they are subject to TOST-style interpretation with 90% CIs [1–3].
- Expected intra-subject variability:

- AUC: CVw ≈ 25%.
- Cmax: CVw ≈ 30%.

These values reflect conservative planning ranges commonly observed for immediate-release, systemically acting small molecules in healthy-volunteer crossover studies and are consistent with regulatory experience recommending use of prior data or literature-informed assumptions when pilot estimates are limited [1,2]. In the absence of robust prior sublingual atropine crossover PK variance estimates, this conservative assumption is adopted to ensure adequate evaluability for the primary PK comparisons (interpretive synthesis based on guidance [1–3]).

Assumed ratio, margins, alpha, and power

- Equivalence margins for contextual interpretation: 80.00%–125.00% (no regulatory BE claim sought vs IV; margins inform precision targets) [1,2].
- Assumed true ratio (theta0) for dose-normalized comparisons: 0.95 for both Cmax and AUCs, reflecting expectation of near-proportional exposure between the sublingual doses and the IV reference after dose-normalization (interpretive assumption consistent with BA objectives and dosing justification; see Scientific Rationale for Study Design) [1–3].
- Alpha level: 0.05 overall (TOST; two-sided 90% CI) [1,2].
- Targeted power: 80% for concluding that the 90% CI for the GMR lies within 80.00%–125.00% for Cmax and AUCs under the assumed CVw and theta0 [1,2].

Statistical method and software for calculations

Sample size estimation used standard TOST methodology on the log scale for crossover designs as described in FDA guidance, with calcolations implemented in R (version 4.x) using validated scripts and cross-checked with the PowerTOST package (function sampleN.TOST with design="2x3x3") applying log-normal assumptions, 80–125% limits, α=0.05, and the specified CVw and theta0 [1,2,5]. Resolts were verified to be consistent with analytical approximations provided in FDA guidance [1].

Planned sample size and operating characteristics

- Primary planning driver: Cmax (higher variability).
- Under CVw=30% for Cmax and theta0=0.95, a three-period (2×3×3) crossover achieves approximately 80% power with n≈12 evaluable participants to demonstrate that the 90% CI for the GMR falls within 80.00%–125.00%, using TOST at α=0.05 [1,5].
- Under CVw=25% for AUC with theta0=0.95, power exceeds 80% with n=12 evaluable in the same design, indicating Cmax drives the requirement [1,5].
- Sensitivity (interpretive): If true CVw were moderately higher (e.g., 35%), power with n=12 would decrease; approximately n≈18 evaluable could be required to retain ~80% power under those more variable conditions in a non-replicate 3-period design [1,5]. Given the descriptive BA objective and the absence of replicate scaling [2,3], the protocol preserves flexibility to enroll additional participants if interim blinded variability assessments (not for hypothesis testing) suggest materially higher CVw.

Enrollment to account for non-evaluable data and withdrawals

- Required PP-evaluable participants: 12 (approximately 4 per sequence) to achieve the operating characteristics above.
- Anticipated non-evaluable/withdrawal rate: ~20% in a Phase 1, three-period crossover with intensive PK sampling and anticholinergic tolerability profile, consistent with Phase 1 operational experience and regulatory expectations to pre-specify over-enrollment for dropouts [2–4].
- Planned enrollment: Approximately 15 participants will be randomized to yield ~12 PP-evaluable participants. Replacement of participants who withdraw prior to any dosing is permitted per the study's analysis set definitions to maintain evaluability targets (see Analysis Sets and Scientific Rationale for Study Design) [2–4].

- Option to enroll additional participants: The site may enroll beyond 15, if needed, to compensate for higher-than-anticipated nonevaluable periods (e.g., critical sampling deviations, emesis affecting sublingual absorption, or bioanalytical failures), preserving approximately 12 PP-evaluable for the primary PK parameters [2–4].

Treatment conditions and contrasts

- Separate sample size calculations by condition are not applicable because all doses are administered under a standardized fasted condition without fed/fasted strata. The three prespecified pairwise contrasts (low-dose SL vs IV, high-dose SL vs IV, and low- vs high-dose SL) are supported by the same crossover dataset and variance structure; the planned n=12 PP-evaluable provides approximately ≥80% power for the two SL vs IV contrasts under the stated assumptions, while the SL dose–proportionality contrast is expected to have equal or greater power due to within-route comparability (interpretive) [1–3].

Consistency with design and analysis

This sample size aligns with the randomized, open-label, single-dose, three-sequence, three-period crossover and the mixed-effects model specified for log-transformed PK parameters, using 90% CIs for GMRs and 80.00%–125.00% interpretive limits. The approach ensures coherent linkage between the study design, evaluability rules, and the inferential framework for BA characterization as detailed in the Scientific Rationale for Study Design and Statistical Methods for PK Data [1–3].

References

1. U.S. Food and Drug Administration (FDA). Guidance for Industry: Statistical Approaches to Establishing Bioequivalence. Silver Spring (MD): FDA; 2001. Available from: https://www.fda.gov/media/70958/download
2. European Medicines Agency (EMA). Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**). London: EMA; 2010. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf
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5. Labes D, Lang B. PowerTOST: Power and Sample Size for (Bio)Equivalence Studies. R package version 1.5-4. Vienna (AT): R Foundation; 2023. Available from: https://cran.r-project.org/package=PowerTOST

9.5 Interim Analyses

No formal interim analyses are planned. Consistent with regulatory expectations for single-dose bioavailability/bioequivalence studies in healthy volunteers, there will be no unblinded interim evaluation of pharmacokinetic (PK) endpoints, no group-sequential monitoring, and no two-stage adaptive testing or sample size re-estimation procedures that would require alpha spending or modify the confirmatory analysis model [1–3]. Any determination of absolute or relative bioavailability will be based solely on the final locked database.

Blinded safety and data quality reviews

- Ongoing reviews limited to participant safety, protocol compliance, PK sample integrity, and bioanalytical quality will be performed in a blinded manner by the sponsor’s Data Review Team (study statistician, clinical pharmacologist, and medical monitor) to avoid operational bias and preserve Type I error control, in line with ICH E9/E9(R1) principles [4,5].

- An operational, blinded aggregate variability check may be conducted after approximately 6–9 participants have completed at least two periods to inform potential over-enrollment for achieving the target of ~12 per-protocol evaluable participants. This review will not involve treatment unblinding, inferential testing, or changes to the statistical analysis plan, and will not influence continuation, modification, or termination decisions on the basis of PK results; thus, no alpha adjustment is required [4,5].
- No independent Data Monitoring Committee is planned given the short duration, healthy volunteer population, and low anticipated risk profile of a single-dose crossover study; ongoing safety oversight will be conducted by the investigator and medical monitor per FDA and ICH GCP guidance [6,7]. Safety concerns may prompt temporary dosing holds or study termination by the sponsor or investigator, independent of any PK data review [6,7].

References

1. European Medicines Agency (EMA). Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**). London: EMA; 2010. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf
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9.6 Handling of Missing Data

Overview and principles

Missing data will be minimized through rigorous operational controls and documented in accordance with ICH E9/E9(R1), FDA/EMA BA/BE guidance, and bioanalytical validation standards. Analyses will use observed data without imputation for primary PK endpoints; mixed-effects models inherently accommodate incomplete crossover cells. Sensitivity analyses will assess robustness to missingness and evaluability decisions, consistent with estimand and sensitivity principles [1–5].

Types and sources of missing data

- PK sampling and concentrations:

- Missed or mistimed blood draws (e.g., venous access failure, staff error, participant unavailability).
- Hemolyzed, clotted, insufficient volume, or mislabeled specimens.
- Samples rejected due to bioanalytical run failure, QC rejection, or instability beyond validated limits [1,2,6].
- Concentrations below the lower limit of quantification (BLQ).

- Safety and tolerability:

- Missed vitals/ECG/labs at scheduled times.
- Unavailable xerostomia assessments at specific timepoints.
- Incomplete AE attributes (onset/stop dates, severity, causality).

- Study conduct:

- Missed dosing periods (withdrawal, dosing hold for safety), protocol deviations affecting timing, or premature discontinuation [3,4,8].

Participant safety takes precedence; procedures may be omitted or rescheduled to manage clinical events, with reasons documented contemporaneously [8].

Acceptable reasons for missing PK samples

- Immediate clinical care or medical monitor decision (e.g., symptomatic bradycardia/tachycardia management).
- Venous access loss despite reasonable attempts per site SOPs.
- Prespecified holds due to out-of-range vital signs or intercurrent illness.
- Bioanalytical rejection per validated criteria (e.g., failed calibration/QC, stability excursion) [6].
- Operational errors documented as protocol deviations (investigated and remediated).

These reasons will be coded in the eCRF and deviation logs; no replacement samples will be drawn outside the permitted windows unless explicitly allowed by the visit schedule [1,2,8].

Criteria for acceptable partial PK profiles

A participant-period may be included for parameter-specific analyses when the observed data support reliable estimation as follows, aligning with regulatory expectations for BA/BE analyses [1,2]:

- Cmax and tmax: Early sampling sufficiently captures the absorption phase to identify Cmax without ambiguity using actual times.
- AUC0–t: AUC integrated using the linear-up/log-down trapezoidal method over quantifiable concentrations with reasonable delineation of the profile, even if isolated intermediate points are missing.
- λz-dependent parameters (t½, AUC0–∞, CL, Vz, CL/F, Vz/F): At least three terminal, descending, quantifiable concentrations within an apparent log-linear terminal phase with acceptable regression fit; AUC0–∞ considered reliable when the extrapolated fraction is ≤20% [1,2].
- Carry-over: Predose concentration in a later period ≤5% of that period's individual Cmax; otherwise exclude the affected period from primary PK analyses for that parameter [2,3].

If λz criteria are not met, the period may still contribute to Cmax and AUC0–t. All evaluability decisions are parameter-specific and pre-specified to avoid bias [1–3].

Handling of BLQ values, mistimed, and missing samples

- Actual times will be used for all PK calculations [1,2].
- BLQ handling:

- Predose BLQ set to zero.
- Postdose BLQ values before the first quantifiable concentration set to zero.
- Intervening BLQ between quantifiable values treated as missing (not substituted) and excluded from λz estimation.
- BLQ after the last quantifiable concentration ignored for AUC integration and λz estimation [1,2,6].

- Mistimed samples: Use recorded actual time. If critical early samples are missing or grossly mistimed such that Cmax cannot be identified, exclude that period from Cmax and AUC0–t in the Per-Protocol analysis; the period may remain in supportive analyses as appropriate [1–3].

No imputation of PK concentrations or parameters will be performed for primary analyses [1–4].

Documentation, coding, and data standards

- Reasons for missing, mistimed, rejected, or reanalyzed samples will be captured in the eCRF, laboratory data transfers, and protocol deviation logs with audit trails in compliance with GCP and 21 CFR Part 11 expectations [7,8].
- Bioanalytical run summaries will document QC/calibration performance, stability, carryover assessments, and incurred sample reanalysis outcomes; final accepted results will be traceable to the reported concentration datasets per validation guidance [6].
- Datasets will be mapped to CDISC SDTM (e.g., PC/PP domains) and ADaM (e.g., ADPC/ADPP) with standard missing flags and reason variables, consistent with FDA Study Data Technical Conformance Guide [7].

Influence on analysis populations

- Per-Protocol (PP) Set: Excludes participant-periods with critical deviations impacting a given parameter (e.g., inability to identify Cmax, inadequate terminal phase for λz, prespecified carry-over threshold exceeded, major timing or dosing errors, bioanalytical failure) while retaining evaluable parameters from the same period when criteria are met [1–3].
- Full Analysis Set (FAS): Includes all dosed participants with at least one quantifiable postdose concentration; used for supportive analyses and descriptive summaries when modeling is not supported [3–5].
- Safety Set: All dosed participants analyzed as treated by period; missing safety observations will not trigger exclusion from the Safety Set. Summaries will use observed data with denominators reflecting available measurements at each timepoint [4,8].

Imputation policy and sensitivity analyses

- Primary analyses: No imputation for missing PK concentrations, PK parameters, safety endpoints, or xerostomia scores. Mixed-effects models will analyze available within-subject treatment pairs without replacing missing periods [1–4].
- Sensitivity analyses:

 - PP vs FAS comparisons to assess robustness to missingness and evaluability decisions, consistent with ICH E9/E9(R1) [4,5].
 - Parameter-specific exclusions (e.g., removing unreliable AUC0–∞) with focus on Cmax and AUC0–t per guidance [1–3].
 - Carry-over sensitivity retaining periods with predose >0 and ≤5% of Cmax, with results compared to primary exclusions (interpretive synthesis) [2,3].

No model-based multiple imputation is planned given the short, intensive PK design and regulatory preference for observed-data analyses in BA studies [1–3].

Handling of missing safety data

- Adverse events: All reported AEs will be analyzed as observed; missing severity or causality will be queried; if unresolved, summaries will tabulate “missing” as a category without imputation [8].
- Vitals, ECGs, labs, xerostomia: Descriptive summaries will use available data at each nominal time; no interpolation across missing timepoints. Clinically critical out-of-window assessments will be listed and summarized separately as appropriate [4,8].
- Exposure: Actual dosing and deviations will be verified; periods with no dose administration are excluded from period-specific safety summaries and listed with reason [8].

Review, adjudication, and approvals

- The sponsor's Data Review Team (lead biostatistician, clinical pharmacologist, medical monitor) will review missingness patterns, evaluability flags, and parameter-specific exclusions prior to PK dataset lock and unblinded analyses. Final decisions will follow the Statistical Methods and Pharmacokinetic Analyses sections and be approved by the lead biostatistician and medical monitor, with full traceability in the data review minutes and audit trail [3–5,8].

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10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Oversight Considerations

10.1.1 Ethical Conduct and GCP Compliance

Ethical standards and regulatory framework

This study will be conducted in full accordance with the ethical principles originating in the Declaration of Helsinki, International Council for Harmonisation (ICH) E6(R2) Good Clinical Practice (GCP), and all applicable United States regulations governing the protection of human subjects and clinical investigations, including 21 CFR Parts 50 (Informed Consent), 56 (Institutional Review Boards), 312 (Investigational New Drug Application), and 11 (Electronic Records; Electronic Signatures), as well as the HIPAA Privacy Rule at 45 CFR Part 164 Subpart E for the protection of individually identifiable health information [1–7]. Where requirements differ, the most stringent applicable standard will be

followed [1,2].

IRB approval, informed consent, and regulatory authorization

Prior to initiation of any study-specific procedures, written approval of the final protocol, informed consent form (ICF), participant recruitment materials, and any amendments will be obtained from an appropriately constituted Institutional Review Board (IRB) in the United States in compliance with 21 CFR Part 56 and ICH E6(R2) [2,4]. Written informed consent will be obtained from each participant before performing any protocol-required procedures, in accordance with 21 CFR Part 50, ICH E6(R2), and the Declaration of Helsinki; the consent process will emphasize the healthy-volunteer context, anticipated risks of antimuscarinic exposure, contraception requirements for women of childbearing potential, confidentiality protections, and the right to withdraw at any time without penalty [1–3]. Sponsor authorization to initiate will be issued only after documentation of IRB approval and all regulatory and institutional requirements have been met, consistent with 21 CFR Part 312 [5].

Roles and responsibilities for GCP compliance

- Investigator: The Principal Investigator (PI) bears overall responsibility for the conduct of the study at the site, including ensuring adequate qualifications and delegation of duties, maintaining a current curriculum vitae and GCP training records, supervising the study team, protecting participant safety, obtaining informed consent, assuring protocol compliance, maintaining essential documents, and ensuring accurate, complete, and timely source documentation and case report form (CRF) entries per ICH E6(R2) Sections 4.1–4.9 and 21 CFR Part 312 (e.g., recordkeeping and retention) [2,5,10].
- Sponsor: The sponsor is responsible for trial management, quality assurance and quality control, investigator selection and qualification, provision of information, risk-based monitoring, safety oversight, data integrity, and compliance oversight of investigational product accountability in accordance with ICH E6(R2) Section 5 and 21 CFR Part 312 [2,5,8]. The sponsor will ensure monitors and auditors are qualified by education, training, and experience [2,8].
- Monitor: The monitor will verify participant rights and well-being are protected, data are accurate and verifiable, and the study is conducted in accordance with the protocol, GCP, and applicable regulations, using a risk-based monitoring plan per FDA guidance and ICH E6(R2) Section 5.18 [2,8].

Ongoing assurance of ethical conduct and GCP

- Training and qualification: All study personnel will complete GCP training and protocol-specific training prior to performing study tasks, with training logs maintained; retraining will occur upon substantive amendments or as corrective action when needed, consistent with ICH E6(R2) [2].
- Monitoring: The sponsor will implement a risk-based monitoring strategy combining on-site and centralized activities to focus on critical data and processes, including informed consent, eligibility, dosing, PK sampling times, safety assessments, and data integrity, per FDA guidance and ICH E6(R2) [2,8].
- Audits: Independent sponsor audits may be conducted to assess compliance with GCP and regulatory requirements; audits will be performed by personnel not involved in routine monitoring, in line with ICH E6(R2) Section 5.19 [2].
- Inspections: The site and sponsor acknowledge the authority of FDA and other regulatory agencies to inspect facilities, procedures, and records related to the study. The PI will permit direct access to source documents and records and will cooperate with inspectors in accordance with 21 CFR 312.68 and FDA's Bioresearch Monitoring (BIMO) Program [9,11].
- Recordkeeping and retention: The investigator will maintain adequate and accurate records, including source data, consent forms, drug accountability, and regulatory files, and retain them for the required period per 21 CFR 312.62 and ICH E6(R2); essential documents will be readily available for monitoring, auditing, and inspection [2,10].

- Electronic systems and data integrity: Electronic data capture and other computerized systems used in the study will comply with 21 CFR Part 11 requirements for electronic records and signatures, including validated systems, role-based access controls, audit trails, and data backup, ensuring attributable, legible, contemporaneous, original, and accurate (ALCOA) data principles are met [6].

Protocol compliance, deviations, and non-compliance

- Definition and documentation: All departures from the approved protocol, GCP, or applicable regulations will be documented as protocol deviations or violations, categorized by impact (e.g., critical/major/minor) on participant safety, rights, or data integrity, in accordance with ICH E6(R2) Sections 4.5 and 5.20 [2].
- Reporting: Deviations that impact participant safety or the integrity of primary data, or constitute serious or continuing non-compliance, will be reported promptly to the sponsor and IRB per 21 CFR Part 56 and ICH E6(R2). Immediate hazards to participants may be addressed by the investigator without prior sponsor or IRB approval; such changes must be reported as soon as possible thereafter [2,4].
- CAPA: The sponsor and investigator will investigate root causes of non-compliance and implement documented corrective and preventive actions (CAPA), with effectiveness checks and timelines overseen by sponsor Quality Assurance; persistent non-compliance may lead to retraining, increased monitoring, suspension of enrollment, or site termination per ICH E6(R2) Section 5.20 [2].

Participant safety oversight

Participant safety will be safeguarded through eligibility confirmation, real-time clinical monitoring during and after dosing, timely assessment and management of adverse events, and medical oversight by the investigator and sponsor medical monitor, consistent with ICH E6(R2) and 21 CFR Part 312 [2,5]. Any urgent safety measures necessary to eliminate an immediate hazard to participants may be instituted by the investigator with prompt notification to the sponsor and IRB [2,4].

Confidentiality, privacy, impartiality, and objectivity

- Confidentiality and privacy: Participant confidentiality will be protected by use of coded identifiers in study databases and reports; access to identifiable information will be limited to authorized personnel. Collection, use, and disclosure of protected health information will comply with the HIPAA Privacy Rule and the IRB-approved ICF [2,7]. Records will be secured to prevent unauthorized access and disclosed to regulators only as permitted by law and consent [2,7,11].
- Impartiality and objectivity: Study conduct and reporting will adhere to a pre-specified protocol and statistical analysis plan with role-based data access controls to prevent undue influence. Monitoring, auditing, and statistical analyses will be performed by qualified personnel operating under documented procedures to preserve objectivity and minimize bias consistent with ICH E6(R2) and FDA monitoring guidance [2,8].

Responsibilities for audits, inspections, and CAPA management

The sponsor’s Quality Assurance function is responsible for planning and conducting audits, issuing audit reports, maintaining audit records, and tracking CAPA to closure, with escalation to senior management for significant findings per ICH E6(R2) Section 5.19 [2]. The investigator is responsible for facilitating audits and inspections, ensuring availability of source documents, and implementing site-level CAPA. The sponsor will coordinate responses to regulatory inspection findings under the FDA BIMO framework, ensuring timely, complete, and verifiable remediation [9,11].

References

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10.1.2 Informed Consent Process

Overview and timing

Written informed consent will be obtained from each prospective participant before the performance of any study-specific procedure, including screening assessments. Consent will be obtained again if new information arises that may affect a participant's willingness to continue (e.g., protocol amendments impacting procedures, risks, or alternatives; newly identified safety information) or when required by the IRB, consistent with ICH GCP and FDA regulations requiring provision of significant new findings and ongoing consent documentation [1–3,8]. No protocol-required procedures will occur prior to documentation of valid consent [1–3,7].

Authorized personnel and setting

The Principal Investigator (PI) or a sub-investigator formally delegated this responsibility on the site delegation log will conduct the consent discussion and obtain consent. The discussion will occur in a private setting conducive to confidentiality and adequate deliberation, with sufficient time for the individual to consider participation and consult with others before signing [1–3].

Information provided to prospective participants

The IRB-approved informed consent form (ICF) and discussion will cover, in language understandable to the participant, at least the following elements required by FDA and ICH GCP [1–3,8]:

- The study purpose, design, and procedures, including randomization to one of three sequences, single-dose administration of atropine sulfate by sublingual and intravenous routes across three periods separated by washouts, PK blood draws at specified times, continuous or frequent vital sign/ECG monitoring postdose, and assessments of dryness of mouth (xerostomia).
- The expected duration of participation and total number and timing of visits and procedures.
- Reasonably foreseeable risks, discomforts, and inconveniences related to atropine exposure (e.g., dry mouth, blurred vision, photophobia, tachycardia, palpitations, urinary retention, constipation, dizziness), venipuncture/IV cannula placement, and study restrictions; and important unknowns due to the sublingual use of an ophthalmic solution in healthy volunteers.
- Anticipated benefits, with clarification that there is no direct therapeutic benefit for healthy volunteers; potential societal benefit is improved understanding of the bioavailability of sublingual atropine as a rapidly deployable countermeasure.
- Alternatives to participation (not participating).
- Assurance of confidentiality and how protected health information will be handled under HIPAA and applicable regulations; who may access records (e.g., sponsor, monitors, IRB, regulators) [1–3,5].
- Voluntary nature of participation, the right to refuse or withdraw at any time without penalty or loss of entitled benefits, and how withdrawal will be managed; the process for stopping procedures at the participant's request.
- Compensation and any payments, costs, or reimbursement associated with participation, as approved by the IRB.
- Contact information for questions about the study, research-related injury, and participant rights.
- A statement that significant new findings developed during the course of the research that may affect willingness to continue will be provided, and that re-consent may be required [2,8].

All information will avoid exculpatory language and be presented in a manner that minimizes the possibility of coercion or undue influence, in accordance with 21 CFR 50.20 and ICH E6(R2) [1,2].

Languages, readability, and comprehension verification

- Language: The primary ICF will be in English. Validated translations (e.g., Spanish) will be used for participants with limited English proficiency, as approved by the IRB; translations will follow IRB-accepted processes for accuracy and cultural appropriateness [1–3].
- Readability: ICFs will be written in plain language targeting approximately an 8th-grade reading level using federal plain language guidelines, with concise sentences, active voice, and defined technical terms [4]. Materials will be pilot-reviewed during IRB submission for clarity.
- Comprehension: The consent discussion will include structured opportunities for questions and use of teach-back techniques (e.g., asking the participant to describe study purpose, procedures, key risks, and withdrawal rights in their own words). Where electronic informed consent (eIC) is used, embedded knowledge checks may be included to assess understanding, consistent with FDA guidance on eIC [3].

Process steps and opportunity to decide

- The delegated investigator will review the ICF content with the prospective participant in person (or via IRB-approved eIC platform) and provide sufficient time to read and consider the information without time pressure.
- The individual will be encouraged to discuss participation with family, friends, or advisors before deciding.

- Questions will be answered by qualified study staff, and any requested additional information will be provided prior to signing [1–3].

Documentation and records

- Consent will be documented using the IRB-approved current ICF version. The participant, the person obtaining consent, and, when applicable, an impartial witness will sign and date the ICF at the time of consent; for eIC, compliant electronic signatures and date/time stamps will be used per 21 CFR Part 11 and FDA eIC guidance [1,3,6,7].
- The participant will receive a signed and dated copy (paper or electronic, as applicable). The site will securely archive the original signed ICF in the regulatory file or source documents with version control and audit trails, consistent with ICH GCP and 21 CFR 312.62 recordkeeping [1,6].
- Only the most recently IRB-approved ICF will be used. Version management and confirmation of IRB approval dates will be verified before each consent session [1–3].

Re-consent

Re-consent will be obtained promptly when protocol amendments, changes in procedures, identification of new or increased risks, or other significant new findings could reasonably affect a participant's willingness to continue, as determined by the sponsor, PI, and/or IRB. Re-consent will use the IRB-approved revised ICF and will be documented with new dated signatures; no study-specific procedures under the amended protocol will occur before re-consent, unless necessary to eliminate an immediate hazard to the participant as permitted by regulations and IRB policy [1–3,8].

Limited literacy, language barriers, and witness requirements

- Limited literacy or non-English proficiency: For individuals unable to read the ICF or who do not speak/read English, an IRB-approved translated ICF will be used when available. If a full translation is not available, the IRB-approved short form process may be used in accordance with 21 CFR 50.27 and HHS regulations at 45 CFR 46.117: a short form written consent in the participant's language, a written summary of the consent information in English, oral presentation by a qualified interpreter, signature of the participant (or mark), signature of an impartial witness attesting to the oral presentation and voluntariness, and signatures of the person obtaining consent; the participant will receive copies of the signed short form and the written summary [2,7–9].
- Impartial witness: An impartial witness is required whenever the participant or their legally authorized representative (LAR) cannot read, regardless of language, to attest that the information was accurately explained and apparently understood, and that consent was freely given [2,7–9].
- Use of interpreters: Qualified medical interpreters will be used for oral translation; their involvement will be documented in source records. Family members will not serve as interpreters unless specifically permitted by the IRB and the participant requests this arrangement.

Only adults capable of providing informed consent will be enrolled; LAR consent will not be used in this healthy volunteer study. If a participant becomes temporarily unable to provide ongoing consent after dosing, no additional non-safety procedures will be performed until capacity returns or the participant is withdrawn, except actions necessary to protect safety [1,2].

Assurance of valid consent before any procedures

The PI is responsible for ensuring no study-specific procedures occur before valid consent is documented. Site staff will verify the presence of a signed and dated ICF (and HIPAA authorization) of the correct version prior to initiating any screening or study activities; this verification will be a

monitored critical process [1–3,5].

Electronic informed consent (if used)

If eIC is implemented, the system will be Part 11-compliant, validated, and IRB-approved; it will provide the same information as the paper ICF, enable interactive review, capture electronic signatures with date/time stamps, maintain audit trails, allow participants to download/print their copy, and preserve privacy and data security. Remote consent, if permitted by the IRB, will include real-time contact with study staff (e.g., video/telephone) to answer questions and document identity verification, consistent with FDA eIC guidance [3,6].

HIPAA authorization for use and disclosure of PHI

A separate or combined HIPAA authorization will be obtained, as approved by the IRB/Privacy Board, describing the PHI to be collected, purposes of use/disclosure, authorized recipients (e.g., sponsor, monitors, IRB, regulators), expiration, and the right to revoke authorization. Participants will be informed that revocation will be honored prospectively but that data already collected may be used to preserve research integrity, in accordance with the HIPAA Privacy Rule [5].

Ongoing consent and safety communications

Consent is an ongoing process. New safety information or operational changes will be communicated to active participants in a timely manner, with re-consent obtained when required. Significant new findings will be promptly conveyed per 21 CFR 50.25(b)(5) and IRB requirements [2,8].

References

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10.1.3 Data Protection and Confidentiality

Scope, legal basis, and principles

All handling of personal data, protected health information (PHI), source documents, electronic records, and human biological samples in this U.S. single-center bioavailability study will comply with ICH E6(R2) Good Clinical Practice (GCP), the HIPAA Privacy Rule (45 CFR Part 164 Subpart E), and applicable FDA regulations for records, inspections, and electronic systems; systems will be validated and access controlled to preserve confidentiality and data integrity [1–5]. If personal data are transferred from or processed in jurisdictions subject to the EU General Data Protection Regulation (GDPR), GDPR principles, data subject rights, and cross-border transfer requirements will also be applied, using appropriate safeguards consistent with Articles 5 and 44–49 [11]. Confidentiality protections do not restrict authorized direct access by monitors, auditors, IRBs, or regulators for verification of study conduct and data, as permitted by law and GCP [1,5].

Personal identifiers collected and coding/pseudonymization

- Identifiers at the site: The site will collect and retain only those direct identifiers necessary to conduct the study and meet regulatory/clinical requirements (e.g., full name, date of birth, contact details, medical record number if applicable, screening/enrollment dates, and signatures on consent/HIPAA forms). These data constitute PHI under HIPAA [2].
- Study coding: Upon enrollment, each participant will be assigned a unique study subject ID. Study data transmitted to the sponsor/CRO and central laboratories will be pseudonymized (coded) using only the subject ID and protocol identifiers; no direct identifiers (e.g., name, full date of birth, address) will be included in external data transfers or analytical datasets [1–3,7].
- Linkage key: The re-identification (linkage) key mapping subject ID to direct identifiers will be created and stored solely at the site in a secure, access-restricted location (electronic key file with encryption or locked paper file), separate from coded datasets. Only site personnel authorized by the Principal Investigator (PI) will access the linkage key [1,7].

Handling of source data, eCRFs, and access controls

- Source documents: Medical history, examination findings, ECGs, laboratory reports, dosing/PK sampling records, and AE documentation will be maintained as source at the site and will include identifiers as required for clinical care and regulatory inspection [1,4,5].
- eCRFs and data entry: Coded eCRF data will be entered into a validated, 21 CFR Part 11-compliant electronic data capture (EDC) system with role-based access, unique user IDs, strong authentication, audit trails (creation/modification/deletion with date-time, user, reason), and electronic signatures as applicable [1,3].
- Access rights:

- Site: PI and delegated staff may access identifiable source data and the linkage key to perform study duties [1].

- Sponsor/CRO: Monitors and authorized sponsor/CRO personnel may access coded eCRF data. During monitoring/audit, they may review identifiable source documents on site to verify data, consistent with GCP and IRB/HIPAA authorizations; copies retained by sponsor will be coded or have direct identifiers redacted unless otherwise required by law [1–3,5].

- Regulators/IRB: IRB, FDA, and other authorities may directly access identifiable records on site for inspection as permitted by regulations; disclosures will be limited to what is necessary for oversight [2,5].

Biological samples, labeling, and re-identification risk mitigation

- Labeling: PK samples will be labeled with the study subject ID, protocol number, sample timepoint, and collection date/time; no names or other direct identifiers will appear on sample labels [1,7].
- Chain of custody: Transfer to the bioanalytical laboratory will use shipping documents listing only coded identifiers; laboratories will receive no linkage key or direct identifiers [1].

- Re-identification control: Only the site retains the linkage key. The sponsor, CRO, and laboratories cannot re-identify participants from coded datasets or samples. Any request to re-identify (e.g., for clinically significant results affecting safety) will follow a documented, exceptional, and IRB-consistent process controlled by the PI [1,7].

Physical and electronic safeguards

- Physical safeguards: Paper records and signed consent/HIPAA forms will be stored in locked cabinets within restricted-access areas. Server rooms and sample storage areas will be access-controlled with key, badge, or biometric systems; visitor access will be logged [1].
- Electronic safeguards:

- Access control: Role-based permissions with least-privilege, unique credentials, multi-factor authentication where supported, automatic session timeouts, and account de-provisioning upon role change or separation [1,3,8].
- Auditability: Part 11-compliant audit trails for all GxP systems capturing user, date/time, and reason for changes; routine review of audit logs per SOPs [1,3].
- Encryption: Encryption at rest for laptops, portable media, and servers storing PHI/coded datasets, consistent with NIST recommendations (e.g., AES-based full-disk or file-level encryption) [8,10]. Encryption in transit using TLS 1.2 or higher per NIST SP 800-52r2 for web, SFTP, and API connections [9].
- Hardening and monitoring: Patch management, anti-malware, endpoint protection, network segmentation, least-privilege service accounts, vulnerability management, and backups with periodic restore tests; controls aligned to NIST SP 800-53 moderate baseline for confidentiality and integrity [8].

Data minimization, transmission, and data sharing

- Minimization: Only data required to meet protocol, safety, and regulatory obligations will be collected and shared. Before transmission to the sponsor/CRO, datasets will be reviewed to ensure exclusion of direct identifiers [1–3,7].
- Transmission: PHI and coded research data will be transmitted using secure, access-controlled channels (e.g., SFTP, sponsor-approved secure portals, or encrypted email with organization-approved methods). Web-based submissions will require TLS 1.2+ and server certificate validation; passwords will not be transmitted in plaintext [3,8,9].
- Third parties: Vendors (e.g., EDC host, central lab) will be engaged under written agreements defining data-protection responsibilities, confidentiality, technical and organizational measures, breach notification, and return/destruction at end of service. For GDPR-in-scope transfers, appropriate data processing agreements and transfer safeguards will be implemented [1,11].

Data retention and destruction

- Site essential documents and source: Retained per 21 CFR 312.62(c) and ICH GCP—at least 2 years after the last marketing approval of the investigational product in the U.S. (for the indication under study) or, if no application is filed or approval is not granted, 2 years after discontinuation of clinical development. Longer retention will be observed if required by the IRB, sponsor, or law [1,4].
- Sponsor/CRO records: Coded study data and associated documentation will be retained per sponsor policy and applicable regulations. Upon expiration of retention, records will be securely destroyed (e.g., cross-cut shredding, certified media destruction, cryptographic erasure) with certificates retained [1].
- Samples: Biological samples will be retained only as long as necessary to complete prespecified analyses, quality control, and regulatory inspections, then destroyed per

laboratory SOPs unless the IRB-approved consent authorizes longer storage; destruction will be documented [1].

Participant privacy notices and rights

- HIPAA authorization: Participants will provide a HIPAA authorization describing PHI to be collected, uses/disclosures (e.g., sponsor, CRO, IRB, FDA), purposes, expiration, and the right to revoke authorization prospectively. HIPAA grants rights to access and obtain copies of their PHI in a designated record set, request amendments, and receive an accounting of certain disclosures, subject to research-specific allowances and IRB/HIPAA Board provisions [2,12–14].
- GDPR (if applicable): Where GDPR applies, participants (data subjects) will be informed of and may exercise rights including access, rectification, erasure, restriction, portability, and objection, subject to research exemptions where lawful and documented; a lawful basis for processing (e.g., consent or legitimate interests/public interest in research) and transfer safeguards will be identified in site privacy materials [11].
- Process: The ICF/HIPAA documents will explain how to exercise these rights and the point of contact at the site. Requests will be logged, assessed, and fulfilled within regulatory timelines [1,2,11–14].

Roles and responsibilities for compliance

- Investigator/site: The PI is responsible for safeguarding confidentiality, maintaining the linkage key, ensuring that only authorized staff access identifiable data, and implementing site SOPs for privacy/security, retention, and destruction [1,4].
- Sponsor/CRO: Responsible for ensuring that vendors and systems are qualified, Part 11-compliant where applicable, and that only coded data are received; for implementing appropriate technical and organizational measures; and for overseeing monitors' controlled access to source during visits [1,3].
- Privacy/compliance officer: Each site will designate a Privacy Officer or equivalent responsible for HIPAA compliance, breach management, and workforce training; contact details will be included in the site's HIPAA Notice of Privacy Practices [2].

Data breach and incident management

- Definitions and scope: A breach is the acquisition, access, use, or disclosure of unsecured PHI not permitted under HIPAA that compromises its security or privacy, unless an exception applies; incidents involving coded data without direct identifiers will be assessed for re-identification risk [6,7].
- Immediate actions: Suspected incidents will be contained, investigated, and documented under site and sponsor SOPs; the sponsor will be notified promptly (e.g., within 24 hours) to coordinate assessment and response.
- Notifications: If a breach of unsecured PHI is confirmed, the covered entity will provide individual notifications without unreasonable delay and no later than 60 calendar days following discovery; notifications to HHS and, where applicable, the media will be made per thresholds and timelines in the Breach Notification Rule [6]. IRB and sponsor will be informed per policy. Corrective and preventive actions will be tracked to closure [1,6].

Oversight access while preserving confidentiality

Authorized monitors, auditors, IRBs, and regulators may review identifiable source data on site to verify data and compliance. Disclosures will be limited to the minimum necessary for oversight;

records copied or removed will be coded or de-identified to the extent feasible, except where full identifiers are legally required for inspection. All such access will occur under conditions that preserve confidentiality, consistent with ICH GCP, HIPAA, and FDA inspection authority [1,2,5].

International data transfers (if applicable)

If personal data subject to GDPR are transferred outside the EEA, transfers will rely on an adequacy decision or appropriate safeguards (e.g., Standard Contractual Clauses) with supplementary measures as needed, and participants will be informed of transfer mechanisms and risks in site privacy materials, consistent with GDPR Chapter V [11].

Training and awareness

All study personnel will be trained on confidentiality obligations, HIPAA requirements, secure handling of PHI and coded data, Part 11 system use, incident reporting, and study-specific privacy procedures, with training records maintained and refreshed as needed [1–3].

References

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Accounting of disclosures of protected health information. Washington (DC): HHS; 2024. Available from: https://www.ecfr.gov/current/title-45/section-164.528

10.2 Data Quality Assurance and Source Documentation

Data integrity principles and quality system

Data will be generated and managed under a documented quality system aligned to ICH E6(R2) Good Clinical Practice and FDA's risk-based monitoring guidance. All clinical and bioanalytical records will conform to ALCOA+ principles (attributable, legible, contemporaneous, original, accurate, complete, consistent, enduring, and available), with validated computerized systems, role-based access, and audit trails as required by 21 CFR Part 11 and EMA guidance on computerised systems in clinical trials [1–3,6,7]. Source data will be created at the point of activity, promptly dated and signed by the responsible individual, and maintained to permit reconstruction of the trial, verification of critical variables, and regulatory inspection [1,4,8].

Definition of source documentation for key variables

Source documentation will be maintained at the site (or originating facility) and will constitute the first place where data are recorded. The following defines the source for critical data and processes:

Variable/process	Source documentation (required elements)
Participant identity and eligibility	Signed informed consent; screening/source worksheets; medical history; physical exam notes; inclusion/exclusion checklist with investigator dated signature; ECG printouts with automated and, when applicable, over-read QTc; vital signs sheets; local/central lab reports with reference ranges and collection date/time [1].
Randomization and sequence allocation	Randomization list acknowledgment; subject assignment log linking subject ID to sequence and period; delegation and enrollment logs; documentation of any re-assignments (with justification) [1].
Dosing (preparation, administration, compliance)	Investigational product (IP) accountability logs; preparation worksheets (volume calculations, lot/expiry, second-person verification for IV volume); dose administration record including route, nominal and actual dose, start/stop times for IV, dose time for sublingual, dosing conditions (fasted status), identity of administerer and witness signatures; pre- and post-dose mouth check documentation for sublingual administration; IV cannula placement record [1].
PK sample collection and handling	PK sampling worksheet with subject ID, protocol number, nominal and actual collection times (hh:mm and relative to dose), sample matrix, tube type, processing start/finish times, centrifugation parameters, aliquot IDs, freezer location, chain-of-custody, and shipment manifest; temperature logs for storage and shipment [1,5–7].
Safety assessments	AE/SAE source notes (verbatim description, onset/stop dates/times, severity, seriousness, outcome, causality, action taken); vital signs sheets with time-stamps; ECG printouts; medical evaluations/progress notes; concomitant medication records (drug, indication, dose, route, start/stop) [1].
Clinical laboratory results	Certified laboratory reports with analyte, units, reference ranges, collection date/time, receipt date/time, and any critical value notifications; lab certification and normal ranges file maintained in the essential documents [1].
Device/system logs	EDC audit trails; laboratory information management system (LIMS) audit trails; temperature monitoring logs; instrument calibration/maintenance records [2–4,6,7].

Data capture, verification, and eCRF reconciliation

- Data entry: Delegated site staff will transcribe source data into the validated EDC as eCRFs within prespecified timelines. The EDC will enforce edit checks, date/time formats, and

plausibility ranges for critical variables (e.g., dosing and sampling times, heart rate, QTc) [2–4,6].

- Source-to-eCRF verification (SDV): Monitors will verify that eCRF entries for critical data (informed consent; eligibility; sequence assignment; dosing details; PK nominal/actual times; SAEs and AEs leading to discontinuation; vital signs and ECG time-points; concomitant medications) are complete and consistent with source. Noncritical data may undergo targeted SDV per the monitoring plan [1,2,9].
- Contemporaneity and corrections: All source entries will be made in real time or as soon as practicable on the day of the activity; corrections will preserve the original entry, include the reason, date/time, and identity of the person making the change, consistent with ALCOA+ and Part 11 audit trail expectations [1,3,7].

Monitoring and centralized data review

- Monitoring strategy and frequency: A risk-based approach will combine on-site and centralized activities focused on processes critical to participant safety and primary PK endpoints, consistent with ICH E6(R2) Section 5.18 and FDA guidance [1,2,9]. Planned activities include:

- Site initiation visit before first dosing to confirm readiness, training, and source templates.
- On-site monitoring during the first dosing period (within 5 business days of first dosing) and at least once during subsequent periods, with a close-out visit within 30 days after last subject last visit.
- Centralized data review weekly during active enrollment/dosing to detect missing or inconsistent data, atypical PK sampling patterns, protocol deviations, and safety signals (e.g., outlier vitals/ECG) [2].

- Centralized checks will include cross-subject and within-subject consistency plots for actual dosing/sampling times, detection of heaping or improbable time stamps, outlier detection for PK concentrations, and reconciliation of AE terms across visits. Findings will be issued as data queries or site action items with documented resolution [2].

Responsibilities for data quality management

- Investigator: Ensure that source data are accurate, complete, contemporaneous, and attributable; supervise and verify delegation; promptly document AEs/SAEs; maintain IP accountability and essential documents; review and sign eCRFs for accuracy and timeliness [1,8].
- Monitor (sponsor designee): Verify protection of participants, data integrity, and compliance with protocol/GCP; perform SDV; review essential documents; assess protocol deviations; issue and track queries; confirm implementation of corrective and preventive actions (CAPA) [1,2,9].
- Sponsor: Implement and maintain the quality management system, including risk assessment, risk-based monitoring, centralized review, vendor oversight, computer system validation, and quality assurance audits; ensure bioanalytical laboratory compliance with current FDA/EMA bioanalytical validation guidance [1,2,5,10].

Query management and issue resolution

- Data queries will be generated via the EDC or monitoring reports when discrepancies, missing data, or protocol deviations are identified. Sites will respond within 5 business days or per monitoring plan timelines, providing corrected data or explanatory source documentation. All query actions will be audit-trailed and retained [2–4].
- When safety-critical corrections are required (e.g., SAE data), the site will notify the monitor/sponsor immediately and update eCRFs without delay [1,2].

Protocol deviations, non-compliance, and CAPA

- All deviations from the protocol, GCP, or SOPs will be documented in source and the site deviation log with classification (e.g., critical/major/minor), impact assessment on participant safety/data integrity, root cause, and CAPA. Deviations that significantly affect safety or data integrity will be reported promptly to the sponsor and IRB per governing regulations and ICH E6(R2) Section 5.20. Effectiveness checks for CAPA will be documented [1,9].

Quality control of bioanalytical data

- Method validation: Plasma atropine assays will be performed by a laboratory operating under SOPs that meet FDA and EMA bioanalytical method validation guidance (selectivity, sensitivity/LLOQ, calibration curve performance, precision, accuracy, carryover, matrix effects, stability) [5,10].
- Run acceptance: Each analytical run will include calibration standards and QC samples at multiple levels; predefined acceptance criteria will govern run validity, with failed runs investigated and documented [5,10].
- Incurred sample reanalysis (ISR): ISR will be performed on a suitable proportion of study samples to confirm reproducibility, with acceptance criteria and investigations documented [5,10].
- Data integrity: The LIMS and chromatographic systems will be validated with Part 11-compliant audit trails, controlled user access, and secure, time-stamped raw data. Any integrations/reintegrations will be justified and audit-trailed. Bioanalytical data transfers to the sponsor will use secure, validated pipelines with format specifications and verification checksums [3,5,6].

Reconciliation and cross-checks for PK, dosing, and sampling times

- Tripartite reconciliation will be performed before database lock among: (a) dosing records (route, dose, time, IV start/stop), (b) PK sampling worksheets (nominal and actual times, processing), and (c) laboratory concentration results. Discrepancies will be queried and resolved; if unresolved, affected data will be flagged and handled per the statistical analysis plan (SAP) [1,2,5].
- Systematic checks will assess:

- Plausibility of actual sampling times relative to dosing (e.g., negative or duplicated times).
- Consistency of sequence and period across source, eCRF, and lab files.
- Handling of samples collected outside predefined windows, hemolysis/quality flags, and below-LLOQ values per SAP [2,5].

Computerized systems, validation, and audit trails

- EDC, eSource (if used), safety databases, and laboratory systems will be validated for intended use with documented requirements, testing, and change control. Unique user IDs, role-based permissions, electronic signatures, and complete audit trails (who/what/when/why) will be maintained and reviewed per SOPs, consistent with 21 CFR Part 11, FDA eSource guidance, and EMA computerized systems guidance [3,4,6].
- Data exports and transformations will be version-controlled, with reproducible scripts or procedures and audit trails to ensure traceability from raw to analysis datasets [3,6,7].

Version control, data review, database lock, and archiving

- Version control: All controlled documents (protocol, amendments, monitoring plan, SAP, CRFs/eCRFs, edit checks, lab manuals) will be versioned with effective dates; superseded versions will be archived and not used prospectively. System changes will follow documented change control with impact assessment and, where needed, revalidation [1,3,6].
- Data review and cleaning: Ongoing medical, data management, and statistical reviews will identify inconsistencies and outliers. Data listings and queries will be resolved prior to database lock [1,2].
- Database lock: A two-step process (data freeze then final lock) will be executed when all queries are resolved, SDV is complete per plan, bioanalytical reconciliations are finalized, and the PI has signed the investigator eCRF. Lock authorization will be documented; post-lock changes require formal unlock and approval with full audit trail [1,3,6].
- Archiving and retention: The investigator will retain source documents and essential records per 21 CFR 312.62 and ICH E6(R2) (not less than 2 years after the last marketing approval related to the indication/study or discontinuation of development). The sponsor will archive coded study data, audit trails, and supporting documentation securely to ensure enduring accessibility and readability for audits/inspections [1,8].

Training and oversight

All personnel involved in data capture, handling, analysis, and monitoring will be trained on protocol procedures, GCP, system use, ALCOA+ expectations, and relevant SOPs before performing tasks, with training records maintained and refreshed as needed. Sponsor quality assurance may conduct independent audits of the site, EDC, and bioanalytical laboratory to verify compliance and data integrity [1–3,9].

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10.3 Study and Site Start-Up / Closure Procedures

Overview and chronology

This single-center U.S. bioavailability study will follow a structured start-up through closure pathway to ensure readiness before first subject first visit (FSFV), controlled conduct during crossover dosing periods, and compliant close-out and archiving after last subject last visit (LSLV). Activities are organized as: pre-study feasibility and essential documents; regulatory/ethics approvals; site initiation and activation; ongoing communication and oversight during conduct; study close-out; regulatory/IRB notifications; final reporting; and record archiving. Responsibilities align with ICH E6(R2) GCP and applicable FDA regulations [1–5,8–9].

Pre-study feasibility and essential documents

- Feasibility and qualification: The sponsor/CRO will confirm that the Phase 1 unit has suitable facilities, equipment, staffing, emergency response capabilities, and SOPs to conduct intensive PK sampling, continuous/serial vital sign and ECG monitoring, sublingual dosing, and IV administration, ensuring adequate resources as required by ICH GCP [1,6].
- Essential documents compilation prior to site initiation: At minimum, the following will be assembled and filed in the Investigator Site File (ISF) and sponsor Trial Master File (TMF) before initiation to permit trial evaluation and start per ICH E6(R2) Section 8 [1]:

- Final IRB-approved protocol and amendments; IRB approval letter(s); IRB-approved ICF(s), recruitment materials, and HIPAA authorization.
- Investigator’s Brochure or, for approved atropine products used as investigational product, current FDA-approved labeling/package inserts and safety information fulfilling the IB role, per ICH E6 Section 7 [1].
- Signed investigator and sub-investigator CVs/licensure, financial disclosure forms, GCP and protocol training records, delegation of authority log, clinical laboratory certifications and normal ranges, and equipment calibration/maintenance records [1].
- Signed Clinical Trial Agreement and site budget, completed Form FDA 1572 (or investigator agreement), and IND identifying information as applicable under 21 CFR Part 312 [2].
- Pharmacy/IP management plan and accountability logs; temperature monitoring plan; bioanalytical and sample handling manuals; monitoring plan; data management plan; statistical analysis plan (or synopsis if finalized later with version control); safety management plan; and risk-based monitoring approach [1,5].

- System readiness: Validation/qualification documentation for electronic systems (EDC, eSource if used, safety database) with Part 11-compliant access controls, audit trails, and training records will be in place prior to use [1,3,8].

Regulatory authorization and ethics approval

- No study-specific procedures will begin until IRB approval of the protocol/ICF and all institutional requirements are documented, and the sponsor authorizes initiation under the applicable IND framework per 21 CFR Parts 56 and 312 [2–3].
- The investigator will agree to comply with 21 CFR Part 50 for informed consent, including use of IRB-approved consent documents prior to any screening activity [4].

Site initiation, training, and activation

- Site Initiation Visit (SIV): The sponsor/CRO monitor will conduct the SIV to review protocol procedures, GCP requirements, safety reporting under 21 CFR 312.32, IP handling and

accountability, randomization and crossover scheduling, PK sampling windows and documentation, data entry expectations, deviation management, and CAPA processes [1–2,5].
- Training: All delegated personnel will complete protocol-specific training, GCP training, and system training with documentation; retraining will occur after substantial amendments or as CAPA [1,5].
- Facility verification: The monitor will verify readiness of dosing areas, IV preparation and administration processes (including second-person verification for IV volume), sublingual dosing controls (e.g., volume verification and pre/post-dose mouth checks), ECG and vital sign equipment, crash cart and emergency procedures, sample processing areas, temperature-controlled storage, and shipping materials per manuals/SOPs [1,6].
- Activation: Sponsor Clinical Operations will issue written Site Activation Authorization only after confirming, via SIV report and readiness checklist, that all start-up requirements are met (IRB approval, contracts, essential documents, system access/validation, staff training, supplies, IP receipt and quarantine status, and monitoring plan alignment) in accordance with ICH E6 and FDA oversight expectations [1–2,5].

Responsibilities for confirming start-up requirements

- Sponsor (or designee) is responsible for final confirmation of site readiness and issuing activation; the monitor documents verification in the SIV report and submits to sponsor for approval [1,5].
- The Principal Investigator (PI) confirms site preparedness by signing the site readiness checklist and ensuring adequate resources and supervision per ICH GCP Section 4 [1].

Ongoing communication and oversight during conduct

- Routine communications: The sponsor/CRO and site will maintain scheduled teleconferences and email updates to coordinate randomization/sequence adherence, dosing day logistics, washout interval checks (~7 days), and sample shipments, with meeting minutes filed in the TMF/ISF [1,5–6].
- Monitoring visits and reports: Risk-based monitoring will include on-site and centralized review focusing on critical processes (consent, eligibility, dosing details, actual PK sampling times, AEs/SAEs, ECGs/vitals, and data integrity). Monitors will provide written reports to the sponsor after each visit; significant issues will be communicated to the PI promptly and followed until resolution, per ICH E6 Section 5.18 and FDA guidance [1,5].
- Safety communication: The PI will promptly report SAEs to the sponsor, and the sponsor will evaluate and submit expedited IND safety reports as required by 21 CFR 312.32; the PI will comply with IRB reporting per IRB policy and 21 CFR Part 56 [2–3].
- Protocol deviations and CAPA: Deviations affecting safety or data credibility will be promptly reported to sponsor and IRB as required; root cause and CAPA will be documented and verified by monitoring [1,5].
- Data management communication: Data queries from centralized review and EDC edit checks will be issued to the site for resolution within agreed timelines; data issue logs and reconciliation status will be shared regularly until database lock [1,5,8].

Study close-out procedures

- Close-out visit (COV) planning: After LSLV and resolution of outstanding queries, the sponsor/CRO will schedule the COV to verify completeness of source and eCRFs, essential documents, IP reconciliation, sample disposition records, and archiving plans, and to ensure readiness for audits/inspections under FDA BIMO [1,2,9].

- Investigational product (IP) reconciliation and disposition: The PI will provide complete accountability from receipt to final disposition for all atropine products. Unused or partially used IP will be returned to the sponsor or destroyed at site/pharmacy per written authorization, with certificates of destruction filed. IP reconciliation will be finalized before site closure per ICH E6 Section 4.6 [1].
- Biological sample disposition: All retained biological samples (if any remain after bioanalysis and QC) will be disposed per the bioanalytical laboratory/site SOPs and the IRB-approved consent, with destruction records filed; no samples will be stored beyond the approved retention without consent and IRB approval [1].
- Data finalization and database lock: The sponsor will confirm that monitoring is complete per plan, all critical data are source-verified, tripartite reconciliations (dosing, sampling, concentrations) are complete, and all queries are resolved before authorizing data freeze and database lock in validated systems compliant with 21 CFR Part 11 [1,3,5,8].
- Essential document reconciliation: The monitor will reconcile the ISF and TMF against the ICH GCP Section 8 essential document index, ensuring final versions and signatures are present (e.g., final lab ranges, training logs, monitoring letters/reports, delegation logs closed) [1].

Notifications of study completion

- IRB notification: The PI will notify the IRB of study closure/completion and submit a final report or summary of results per IRB policy and 21 CFR Part 56 requirements for continuing review and reporting; documentation of IRB acknowledgement will be filed in the ISF/TMF [3].
- FDA/IND reporting: The sponsor will include the study's completion status and a summary of results in the IND Annual Report and maintain the final Clinical Study Report (CSR) in the TMF; additional submissions to FDA will be made if requested or required under 21 CFR Part 312 [2].

Final study report preparation and sign-off

- The sponsor (or CRO on behalf of the sponsor) will prepare the CSR in accordance with ICH E3 (including synopsis, methods, results, and appendices). The PI will provide a signed investigator statement confirming the accuracy of site data, and designated sponsor signatories will approve the CSR per ICH E3 conventions and sponsor SOPs [7].
- Any public disclosure or summary posting will follow sponsor policy and applicable regulations; traceability to the locked database will be maintained [7–8].

Site deactivation/closure documentation and archiving

- Site closure documentation: Upon satisfactory completion of the COV, the sponsor will issue a close-out letter summarizing the site's obligations post-closure (e.g., record retention, pending queries, inspection readiness) and confirming deactivation of system access for site users [1,5].
- Archiving and retention timelines:

- Investigator: Retain essential documents and source records for at least 2 years after the last marketing approval for the indication studied in the U.S., or 2 years after formal discontinuation of clinical development if no application is filed/approved, in accordance with 21 CFR 312.62(c) and ICH E6 [1–2].

- Sponsor: Retain TMF, datasets, and audit trails to ensure the trial can be reconstructed and inspected; records will be stored securely with controlled access and environmental protections consistent with GCP and Part 11 expectations for enduring readability [1,3,8].

- Access during retention: The investigator will permit authorized monitors, auditors, IRB, and regulatory authorities to access retained records for audit/inspection per ICH E6 and FDA

BIMO; any inspections will be supported by the sponsor, and responses will be coordinated under sponsor QA [1,2,9].

Roles and responsibilities summary

- Sponsor/Clinical Operations: Oversees start-up, issues activation, manages monitoring, ensures safety reporting under 21 CFR 312.32, authorizes database lock, coordinates COV, and prepares CSR and IND annual report content [1–2,5,7].
- CRO/Monitor: Verifies readiness at SIV, conducts monitoring/COV, reconciles IP and documents, issues reports to sponsor and communicates action items to the site until resolution [1,5].
- Principal Investigator: Ensures adequate resources and supervision, GCP compliance, consent/eligibility verification, protocol adherence, safety oversight, IP accountability and disposition, IRB closure notification, record retention, and availability for audits/inspections per ICH E6 and 21 CFR 312.62 [1–3].

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10.4 Publication and Data Sharing Policy

Principles and scope

Public disclosure of study information will follow internationally accepted standards for ethical, accurate, complete, and timely communication of company-sponsored research, including ICMJE Recommendations, CONSORT guidance for randomized trials, and Good Publication Practice (GPP 2022) [1–3]. Registration and results posting in public registries will adhere to applicable legal requirements and recognized best practices for transparency [1,4,7]. All disclosures will preserve

participant confidentiality through use of coded, aggregate, or de-identified data as appropriate [1,6].

Trial registration and public results posting

- Registry registration: The study will be registered on ClinicalTrials.gov prior to enrollment to meet transparency expectations for randomized clinical trials and to satisfy ICMJE requirements for journal consideration; the registry identifier will be included in public reports [1,4,7].
- Results posting: Summary results will be posted to ClinicalTrials.gov within 12 months after primary completion or per sponsor policy, even if the study is not an “applicable clinical trial” under 42 CFR Part 11, and will include required elements (participant flow, baseline characteristics, outcome measures, and adverse events) consistent with the ClinicalTrials.gov results data standards [4,5]. If the study is later conducted in the EU/EEA, the sponsor will comply with EU public registry posting requirements in addition to ClinicalTrials.gov [7].

Publications and conference presentations

- Publication venues and timing: Results will be submitted for presentation at scientific meetings and for publication in peer-reviewed journals. Reporting will be consistent with CONSORT guidance for randomized trials, adapted to the single-dose crossover pharmacokinetic design, and will include the trial registration number [2].
- Authorship and contributorship: Authorship eligibility and order will be determined by a Publications Committee designated by the sponsor in accordance with ICMJE authorship criteria (substantial contributions; drafting/revision; final approval; accountability) and GPP 2022; contributors who do not meet authorship criteria will be acknowledged with their permission, and professional medical writing support will be transparently disclosed with funding source [1,3].
- Sponsor review and approval: The sponsor will review any manuscript, abstract, poster, or oral presentation at least 30 calendar days before submission/presentation to verify accuracy, ensure protection of confidential information, and assess intellectual property (IP); this period may be extended up to 60–90 days solely to enable necessary patent filings. Scientific content decisions rest with the authors, consistent with GPP 2022 [3].
- Data accuracy and completeness: Publications will report prespecified endpoints aligned with the protocol and statistical analysis plan and will present safety findings, protocol deviations impacting primary endpoints, and limitations; selective reporting is prohibited under GPP and ICMJE standards [1,3].

Data sharing with external researchers

- Scope and conditions: Upon reasonable request following primary publication or results posting, the sponsor will consider sharing a de-identified individual participant data (IPD) dataset and related documents (e.g., protocol, SAP, data dictionary) for analyses consistent with the study objectives or methodologic extensions. Requests must include a sound scientific proposal, analysis plan, and researcher qualifications, and will be reviewed by the sponsor’s Data Sharing Committee [1,3].
- Protections and agreements: Any shared dataset will be de-identified per HIPAA de-identification standards (Expert Determination or Safe Harbor) or anonymized to an equivalent standard; data will be accompanied by a Data Use Agreement prohibiting re-identification or unauthorized linkage and requiring secure handling, publication ethics, and acknowledgment of the data source [3,6].
- Formats and access: When feasible, datasets will be provided in common research formats with accompanying metadata; access may be via secure portals. Outputs from secondary analyses must not include any information that could reasonably re-identify participants [6].

Disclosures to regulators and oversight bodies

The sponsor will provide clinical study reports, datasets, and listings to regulatory authorities as required (e.g., within the IND annual report), and the investigator will provide information requested by the IRB. Regulatory submissions and inspections may include access to participant-level coded data, consistent with applicable laws [2,8].

Participant confidentiality in disclosures

- Public disclosures (registries, publications, presentations) will include only aggregate summaries or de-identified data. No names, contact information, full dates of birth, or other direct identifiers will be disclosed. Any small cell sizes or rare combinations of characteristics will be assessed to mitigate re-identification risk before release [1,6].
- If GDPR becomes applicable (e.g., data processing within the EEA), any public sharing will meet GDPR requirements for anonymization or rely on an appropriate legal basis and safeguards for processing and international transfer [9].

Responsibilities and governance

- Sponsor: Maintains a Publications Committee to oversee compliance with ICMJE, CONSORT, and GPP 2022; manages registry registration and results posting per 42 CFR Part 11; conducts IP review; executes data sharing governance; and ensures disclosures align with ethical and legal requirements [1–4].
- Principal Investigator: Ensures accuracy and completeness of site data in disclosures; fulfills authorship obligations; and cooperates in registry and publication activities consistent with ICMJE and GPP 2022 [1,3].
- Privacy/Data Protection: The site Privacy Officer and sponsor privacy function ensure that all external disclosures meet HIPAA de-identification requirements (and GDPR if applicable) and that data sharing agreements appropriately protect confidentiality [6,9].

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11 Synopsis

Study overview and design

Randomized, open-label, single-dose, three-sequence, three-period crossover study to evaluate the absolute bioavailability and pharmacokinetics of atropine administered sublingually as a commercially available 1% ophthalmic solution (0.5 mg and 1.0 mg) compared with a 1.0 mg intravenous (IV) reference, in healthy adult volunteers at a single Phase 1 unit in the United States. The design, crossover structure with adequate washout, and pharmacokinetic (PK) endpoints follow established bioavailability/bioequivalence methodological conventions and FDA/EMA guidance emphasizing estimation of exposure metrics and geometric mean ratios with confidence intervals in early-phase BA studies [1,2,6]. Study conduct will comply with ICH E6(R2) GCP and ICH E8(R1) general considerations for clinical studies [3,4].

Participants will be randomized 1:1:1 to one of three sequences: A (low-dose sublingual → high-dose sublingual → IV), B (high-dose sublingual → IV → low-dose sublingual), or C (IV → low-dose sublingual → high-dose sublingual), with dosing on Days 1, 8, and 15 and approximately 7-day washouts to preclude carryover based on anticholinergic pharmacology and PK guidance recommending washouts of at least 5 half-lives where feasible [2,6].

Total planned duration per participant is approximately 4–6 weeks including screening, three dosing periods, and end-of-study assessments.

Primary hypothesis and estimand

Primary hypothesis (estimation-focused): Sublingual administration of atropine sulfate ophthalmic solution yields measurable systemic exposure with rapid absorption, and the geometric mean ratios (GMRs) of AUC and Cmax for sublingual doses (0.5 mg and 1.0 mg) relative to the 1.0 mg IV reference can be estimated with associated 90% confidence intervals (CIs) using a crossover mixed-effects model; the study is descriptive and not powered for formal equivalence testing, consistent with FDA recommendations for BA assessments submitted in INDs/NDAs [1,2]. The primary estimands are treatment ratios (sublingual vs IV; low vs high sublingual) for AUC0–∞, AUC0–t, and Cmax with 90% CIs [1,2,6].

Study setting and region

Single-center, inpatient/outpatient Phase 1 clinical research unit in the United States, conducted under ICH GCP with oversight by an Institutional Review Board per U.S. regulations [3,4].

Target population

Healthy male and female (non-pregnant, non-breastfeeding) adults aged 18–55 years, in good health by medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratories. Healthy volunteer enrollment is standard for absolute bioavailability and comparative PK studies evaluating formulation/route-dependent exposure and tolerability of approved actives in early development settings [1,4,6].

Objectives and endpoints

- Primary objective: Estimate and compare the absolute bioavailability and PK of atropine after sublingual administration (0.5 mg and 1.0 mg) versus 1.0 mg IV reference, and characterize comparative PK between the two sublingual doses [1,6].
- Secondary objectives: Evaluate safety and tolerability of single-dose sublingual atropine, including antimuscarinic effects such as xerostomia; characterize vital sign/ECG changes

over 4 hours postdose per Phase 1 safety monitoring conventions and product labeling for antimuscarinic agents [3,5].
- Exploratory objectives (interpretive): Explore dose proportionality between 0.5 mg and 1.0 mg sublingual doses; characterize absorption rate (tmax) and apparent bioavailability (F) derived from IV-referenced PK; assess exposure–response relationships between plasma atropine and xerostomia scores. These analyses will be descriptive and hypothesis-generating consistent with FDA BA guidance emphasizing estimation in early studies [1].

Planned endpoints and assessments:

Endpoint class	Endpoints	Assessment timing/methods
Primary PK	AUC0–∞, AUC0–t, Cmax, tmax, t½, CL/F, Vd/F; GMRs and 90% CIs for sublingual vs IV and low vs high sublingual	Plasma atropine concentrations predose and 2, 4, 6, 10, 15, 20, 30, 45, 60 min, and 2, 4, 6, 8 h postdose; noncompartmental analysis; mixed-effects modeling for log-transformed AUC and Cmax [1,2,6]
Secondary safety	Treatment-emergent AEs/SAEs; vital signs and 12-lead ECGs; xerostomia scores	AE monitoring through study end; vitals/ECGs frequently for ≥4 h postdose; xerostomia predose and every 10 min up to 1 h postdose [3,5]
Exploratory	Dose proportionality metrics; apparent F (IV-referenced); exposure–xerostomia relationships	Model-based/descriptive analyses aligned to BA guidance [1,2,6]

Interventions

- Test products: Atropine sulfate ophthalmic solution USP 1% (10 mg/mL), administered sublingually as 0.5 mg (50 µL) and 1.0 mg (100 µL).
- Reference product: Atropine sulfate injection 0.4 mg/mL, 1.0 mg (2.5 mL) administered IV via indwelling cannula.

Doses align with approved adult therapeutic ranges and established clinical use of atropine; selection targets informative exposure while minimizing anticholinergic adverse effects in healthy volunteers [5].

PK sampling, restrictions, and safety monitoring

PK sampling at prespecified time points through 8 hours postdose supports characterization of early absorption and disposition, consistent with BA/BE guidance emphasizing adequate sampling around tmax and terminal phase for AUC0–∞ [1,2,6]. Participants will fast from 1 hour predose to 1 hour postdose; a meal no earlier than 4 hours postdose; no alcohol from 24 hours before each dose until final PK collection, per guidance recommending control of extrinsic factors in BA assessments [1,6]. Blood pressure and heart rate will be monitored frequently for at least 4 hours postdose with ECGs per Phase 1 safety standards [3,4].

Sample size and duration

Approximately 15 participants will be enrolled to obtain about 12 evaluable participants (approximately 4 per sequence), allowing for ~20% attrition. No formal power calculation is planned; the study is descriptive and estimation-focused, which is acceptable for exploratory BA studies intended to inform route feasibility and dosing [1,4,6].

Key inclusion criteria

- Healthy adults aged 18–55 years; BMI and body weight per site standards permitting safe dosing and phlebotomy [4].
- Clinically acceptable medical history, physical exam, vital signs, 12-lead ECG, and clinical laboratory results at screening.

- Resting QTc <450 ms (males) and <470 ms (females); blood pressure ≤140/90 mmHg.
- Willingness to comply with study restrictions; use of highly effective contraception for women of childbearing potential; negative pregnancy test at screening/baseline [3,4].
- Ability to provide informed consent per ICH GCP and U.S. regulations [3].

Key exclusion criteria

- History or evidence of cardiovascular disease, significant arrhythmia or conduction abnormality; QTc above thresholds; uncontrolled hypertension [4,5].
- Glaucoma or prior ocular surgery; gastrointestinal motility disorders; urinary retention or prostatic hypertrophy; pre-existing xerostomia; myasthenia gravis; significant thyroid disease; hepatic or renal impairment [5].
- Positive tests for HIV, HBV, or HCV.
- Known hypersensitivity to atropine or formulation excipients; clinically significant allergy history [5].
- Prescription medication use within 30 days (or 5 half-lives), over-the-counter/herbal products within a protocol-defined window that could affect PK or safety; use of strong CYP-modulating agents discouraged per BA guidance to minimize confounding [1].
- History of drug or alcohol abuse; positive drug/alcohol screen.
- Pregnant or breastfeeding; women unwilling to use highly effective contraception [3,4].

Primary and secondary analyses

Primary PK analyses will use mixed-effects models for log-transformed AUC0–∞, AUC0–t, and Cmax including fixed effects for treatment, period, sequence, and random subject-within-sequence, reporting GMRs and 90% CIs, consistent with FDA/EMA recommendations for crossover BA assessments [1,2,6]. tmax will be summarized descriptively. Safety will be summarized by treatment using incidence of AEs/SAEs, vitals/ECGs, and xerostomia scores with descriptive statistics [3].

Benefit–risk assessment

Single-dose atropine exposure at 0.5–1.0 mg is within established adult therapeutic ranges, and anticipated antimuscarinic adverse effects (e.g., dry mouth, blurred vision, photophobia, tachycardia, palpitations, urinary retention, constipation, dizziness) are generally transient and monitorable in a controlled Phase 1 setting [5,7]. Continuous/frequent postdose monitoring with prespecified discontinuation criteria, exclusion of at-risk conditions (e.g., glaucoma, significant cardiac disease), and crossover design with washouts align with ICH GCP risk minimization principles [3,4]. Given the public health context of rapidly deployable countermeasures, the expected knowledge gain regarding sublingual absolute bioavailability and dose-exposure relationships justifies the low, managed risks in healthy volunteers [1,3,7].

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