Study Title: Clinical Study to investigate the effect of administration of selective serotonin reuptake inhibitors and an opioid on ventilation

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CLINICAL STUDY PROTOCOL

Clinical study to investigate the effect of administration of selective serotonin reuptake inhibitors and an opioid on ventilation

PROTOCOL NO. SCR-012

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CONFIDENTIAL

SPONSOR SIGNATURE PAGE

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- International Council for Harmonisation (ICH) harmonised tripartite guideline E6 (R2): Good Clinical Practice; and
- All applicable laws and regulations, including without limitation, data privacy laws and compliance with appropriate regulations, including human subject research requirements set forth by the Institutional Review Board (IRB).

DocuSigned by:
David Strauss
Signer Name: David Strauss
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Signing Time: 27-Jul-2022 | 11:02:36 AM PDT
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27-Jul-2022

David Strauss, MD, PhD
Director, Division of Applied Regulatory Science
U.S. Food and Drug Administration

Date

INVESTIGATOR SIGNATURE PAGE

I confirm that I have read and that I understand this protocol, the investigator brochure, and other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- ICH harmonised tripartite guideline E6 (R2): Good Clinical Practice;
- All applicable laws and regulations, including without limitation data privacy laws and regulations;
- Human subject research requirements set forth by the IRB;
- Regulatory requirements for reporting of serious adverse events (SAEs) defined in Section 4.7.3.1 of this protocol; and
- Terms outlined in the Clinical Study Site Agreement.

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Section 6 of this protocol.

DocuSigned by:
Jan Matousek
Signer Name: Jan Matousek
Signing Reason: I have reviewed this document
Signing Time: 27-Jul-2022 | 11:05:49 AM PDT
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Jan Matousek, D.O. Principal Investigator	27-Jul-2022 Date

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PROTOCOL SYNOPSIS

Protocol Number:	SCR-012
Title:	Clinical study to investigate the effect of selective serotonin reuptake inhibitors and an opioid on ventilation
Investigators:	Principal Investigator: Jan Matousek Study Physician: Jan Matousek
Study Phase:	1
Study Period:	This is a 3-period, double-blind, randomized crossover study. The duration of each treatment period will be 21 days in addition to a three-week washout between periods. The duration of study participation will be 106 days (excluding the screening period).
Study Site:	Spaulding Clinical Research Unit, West Bend, Wisconsin
Background and Motivation:	A well-known and potentially fatal adverse reaction associated with opioid administration, particularly in the scenario of misuse, abuse, or when coadministered with certain other drugs is that people 'stop breathing'. Research suggests this is caused by a reduced ventilatory response to increasing levels of carbon dioxide. In August 2016, the U.S. Food and Drug Administration (FDA) included boxed warnings about increased potential for respiratory depression with co-use of benzodiazepines and opioids.[1] In response to this action, concerns were raised because patients may be prescribed other psychotropic drugs that may have similar adverse reactions when combined with opioids. Subsequently, FDA performed a review of the literature and identified 13 psychotropic drugs for evaluation in an in vivo animal study (using a rat model). The in vivo study identified 4 drugs that had significant effects when combined with oxycodone on arterial carbon dioxide.[2] Two drugs (quetiapine and paroxetine) were selected for further evaluation in a human clinical study evaluating drug effects on hypercapnic ventilation. To study the effects of opioids alone or in combination with other psychotropic drugs on ventilation, FDA used a procedure referred to as Read rebreathing.[3-4] With Read rebreathing, study participants rebreathe through a circuit with increased levels of O_2 and CO_2 . The artificially increased levels of CO_2 trigger the subjects to increase ventilation. This "hypercapnic ventilatory response" can be decreased by opioids, benzodiazepines, and other drugs.[5-19] The previous study, which included 5-days of study drug dosing with oxycodone administered on day 1 or 5 showed quetiapine co-

	administered with oxycodone did not cause a significant decrease in hypercapnic ventilation compared to oxycodone alone.[20] In contrast, paroxetine caused a decrease in hypercapnic ventilation compared to oxycodone alone, and an additive effect was seen when combined with oxycodone. This mechanism is due to a direct pharmacodynamic effect, rather than a pharmacokinetic interaction and most likely due to its serotonergic properties. Paroxetine is highly selective for inhibiting the serotonin transporter versus other monoamine transporters and other receptors in general.[21-22] Although different types of serotonergic receptors and neurons can have differential effects on breathing, paroxetine does not bind to serotonin receptors at clinically-relevant therapeutic exposures.[23-24] In addition, nonclinical and clinical studies have shown different SSRIs can have independent effects on ventilation, though SSRIs have not been shown to cause severe respiratory depression on their own.[25-43] This supports the need for additional investigation on the effects of serotonergic drugs. The previous study evaluated effects on ventilation over 5-days of dosing. SSRIs take approximately 3 weeks to reach maximal therapeutic effect there, which correlates with the time required for pre-synaptic inhibitory serotonergic receptors to desensitize.[44-46] Drug-effects on ventilation should be evaluated for a similar time period to confirm if effects persist under steady state conditions As part of this proposed study with a longer dosing duration and different SSRIs, hypercapnic ventilation will be assessed under hyperoxic and hypoxic gas levels using a modified Read rebreathing approach referred to as Duffin rebreathing. These modifications allow for critical physiological measurements and thresholds to be captured that can then be used to model the effects of drugs when there are dynamic changes in PO_2 and PCO_2, such as during a real-world opioid overdose.[47-52]
Objectives and Endpoints:	The objectives of this study are: **Primary Objectives** Primary objectives include the following: • To study ventilatory effects of SSRIs (paroxetine or escitalopram) combined with oxycodone compared to oxycodone alone after 21 days of SSRI dosing • To study ventilatory effects of SSRIs (paroxetine or escitalopram) compared to placebo after 20 days of SSRI dosing **Secondary Objectives** Secondary objectives include the following:

	• To compare ventilatory effects of SSRIs (paroxetine or escitalopram) combined with oxycodone compared to oxycodone alone after 6 and 12 days of SSRI dosing • To compare ventilatory effects of SSRIs (paroxetine or escitalopram) compared to placebo after 5 and 11 days of SSRI dosing **Exploratory Objectives** • To study whether paroxetine or escitalopram affects the pharmacokinetics of oxycodone • To evaluate differences in hypercapnic ventilatory response under hyperoxic or hypoxic rebreathing • To study whether there is a direct pharmacodynamic interaction between paroxetine or escitalopram and oxycodone • To summarize additional pharmacokinetic parameters and pharmacodynamic measurements collected during the study The endpoints for this study are: **Primary Endpoint** • Minute ventilation at the 55mm Hg end tidal CO_2 point (VE55) under hyperoxic conditions on day 21 • VE55 under hyperoxic conditions on day 20 **Secondary Endpoints** Secondary endpoints include the following: • VE55 under hyperoxic conditions on days 6 and 12 • VE55 under hyperoxic conditions on days 5 and 11 **Exploratory Endpoints** • The maximum observed plasma concentration (C_{max}) and area under the plasma concentration time curve (AUC) of oxycodone and oxymorphone on days 6, 12, and 21 • Baseline minute ventilation, ventilatory recruitment threshold, slope of the minute ventilation / end-tidal partial pressure of carbon dioxide (end-tidal PCO_2) regression line, and extrapolated ventilatory recruitment threshold collected during hyperoxic and hypoxic rebreathing • The PK/PD relationship for study drugs when administered alone or in combination with oxycodone • Additional pharmacokinetic and pharmacodynamic (e.g., ventilation, pupillometry, ECG) endpoints as specified in the protocol

Study Design:

This study will be a randomized, double blind, three period crossover study with approximately 25 healthy volunteer participants. This study will include three 21-day treatment periods with a 3-week washout between each period. Each subject will be randomized to 1 of 6 treatment sequences (i.e., ABC, ACB, BAC, BCA, CAB, CBA). FDA will prepare the randomization schedule.

Study Schedule:

Day -1	**Days 1-21**	**Days 22-42**	**Days 42-63**	**Days 64-84**	**Days 85-105**	**Day 106**
Check-in	Period 1	Washout	Period 2	Washout	Period 3	Check-out

Study Treatments:

Treatment	**Day**					
	1-5	**6**	**7-11**	**12**	**13-20**	**21**
A	Placebo QD	Placebo + 10 mg oxycodone	Placebo QD	Placebo + 10 mg oxycodone	Placebo QD	Placebo + 10 mg oxycodone
B	40 mg paroxetine QD	40 mg paroxetine + 10 mg oxycodone	60 mg paroxetine QD	60 mg paroxetine + 10 mg oxycodone	60 mg paroxetine QD	60 mg paroxetine + 10 mg oxycodone
C	20 mg escitalopram QD	20 mg escitalopram + 10 mg oxycodone	30 mg escitalopram QD	30 mg escitalopram + 10 mg oxycodone	30 mg escitalopram QD	30 mg escitalopram + 10 mg oxycodone

Subjects will report to the study site for screening from Days -28 to -2. During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent form will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure he or she has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form before any study-specific procedure is performed, including randomization.

After the consent process is complete, demographic data, medical history, and concomitant medications will be recorded. A physical examination will be performed by a study team member. Clinical laboratory tests (hematology, seum chemistry, and urinalysis) will be performed. Female subjects must have a negative pregnancy test result. Screening tests will be performed within 28 days of and no later than 2 days before Day 1.

At check-in for the first period (Day -1), eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented. At check-in for all periods, vital sign

	measurements, and a 12-lead electrocardiogram (ECG) will be performed. In addition, clinical laboratory, drug, alcohol, two rebreathing assessments (one hyperoxic and one hypoxic), and a COVID-19 rapid antigen. An intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed). Participants will enter the clinic on a staggered basis in cohorts of approximately five so that no more than five subjects are undergoing rebreathing on any given day. Subjects will be staggered to allow for direct safety overview by medical staff during Duffin's rebreathing. Participants will receive either placebo, paroxetine, or escitalopram on days 1-21 for each period. Oxycodone will be administered on days 6, 12, and 21 of each period. Dosing of the study drugs will occur at time 0 on each day. Oxycodone dosing will be at 3 h so that maximum concentration occurs for all drugs is at approximately the same time. Participants will be confined to the study clinic from day -1 until the first day of each washout period. There will be a three-week washout between each period. Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days. Upon return to clinic, on Days 42 & 84, eligibility criteria to continue study participation will be reviewed (see Schedule of Events for criteria listed on Days 42 & 84), any changes in medical history (including concomitant medications) will be documented.
Subject Population:	Approximately 25 healthy participants are planned for enrollment. Every effort will be made to maintain an approximate 50:50 male-to-female sex distribution.
Study and Reference Drugs, Dosage, and Route of Administration:	For all drugs, standard drug doses or doses used in prior studies in healthy participants will be used in this study. Multiple doses of the assigned study drug will be administered to each subject during the treatment period. Oxycodone, paroxetine, escitalopram, ondansetron, and placebo will be administered orally. Subjects will complete each of the three treatments (A, B, and C) and will receive the following drugs alone or in combination: • Oxycodone 10 mg QD (2 x 5 mg tablets) on days 6, 12, and 21 in each treatment (A-C) • Paroxetine 40 mg QD (2 x 20 mg tablet) on days 1-6 and 60 mg QD (3 x 20 mg tablets) on days 7-21 in treatment B

	• Escitalopram 20 mg QD (2 x 10 mg tablet) on days 1-6 and 30 mg QD (3 x 10 mg tablets) on days 7-21 in treatment C • Matching oral placebo for paroxetine and escitalopram • Participants will also receive ondansetron 4 mg orally 30 min before oxycodone administration
Inclusion Criteria:	Subjects who meet all the following inclusion criteria will be eligible to participate in the study: 1. Subject signs an IRB approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization) before any study related procedures are performed. 2. Subject is a healthy, non-smoking man or woman, 18 to 50 years of age, inclusive, who has a body mass index of 18.5 to 33.0 kg/m^2, inclusive, at Screening. 3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, pulse oximetry, 12-lead ECG results, and physical examination findings at screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee). 4. Subject must have a negative test result for alcohol and drugs of abuse at screening and check-in days. 5. Subject must test negative for severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) by a rapid antigen test at check-in for all study periods. If a subject's test comes back as invalid, the test can be repeated. 6. Female subjects must be of non-childbearing potential (confirmed with follicle-stimulating hormone levels > 40 mIU/mL) or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before check-in (Day -1) and agree to remain strictly abstinent for the duration of the study and for at least 1month after the last application of study drug; OR 2) be practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before check-in (Day -1) until at least 1 month after the end of the study. 7. Male subjects must agree to practice 2 highly effective methods of birth control (as determined by the investigator or designee) from at least 1 month before check-in (Day -1) until at least 1 month after the last dose of study drug. 8. Subject is highly likely (as determined by the investigator) to comply with the protocol defined procedures and to complete the study.
Exclusion Criteria:	Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

	1. Subject has used any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer) or complementary and alternative medicines within 28 days before the first dose of study drug. This includes prescription or nonprescription ophthalmic drugs. 2. Subject is currently participating in another clinical study of an investigational drug or has been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound. 3. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff, electronic cigarettes) within 6 weeks of Screening. Subjects must refrain from using these throughout the study. 4. Subject has consumed alcohol, xanthine containing products (e.g., tea, coffee, cola), caffeine, grapefruit, or grapefruit juice within 24 h of check-in. Subjects must refrain from ingesting these throughout the study. 5. Subject has a history or evidence of a clinically significant disorder, condition, or disease (e.g., cancer, human immunodeficiency virus [HIV], hepatic or renal impairment) that, in the opinion of the investigator would pose a risk to subject safety or interfere with the study evaluation, procedures, or completion. This includes subjects with any underlying medical conditions that put subjects at increased risk of severe illness from COVID-19 based on the Centers for Disease Control and Prevention (CDC) guidelines. 6. Subject has any signs or symptoms at screening or check-in of any study period that are consistent with COVID-19. Per current CDC recommendations this includes subjects with the symptoms cough or shortness of breath or difficulty breathing, or at least two of the following symptoms: fever, chills, repeated shaking with chills, muscle pain, headache, sore throat, or new loss of taste/smell. In addition, the subject has any other findings suggestive of COVID-19 risk in the opinion of the investigator. 7. Subject has known or suspected allergies or sensitivities to any study drugs. 8. Subject has clinical laboratory test results (hematology, serum chemistry and urinalysis) at Screening or period check-In that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator. Clinical laboratory results may be repeated once, as needed, for confirming results at Screening and period check-in. 9. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.

	10. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access. 11. Female subject is currently pregnant or lactating or was within 3 months of study enrollment. 12. Subject has a history of opioid or psychotropic drugs within 60 days of the start of the study. 13. Subject has a history of asthma that has required medication within the last five years. 14. Subject has non-reactive or mishappen pupil(s) or damaged orbit structure or surrounding soft tissue is edematous or has an open lesion. 15. Subject has a Mallampati score of >2. 16. Subject's Duffin rebreathing data is of poor quality or subject does not agree to remain clean-shaven for all days when the Duffin rebreathing procedure is performed. 17. Subject has a history of sleep disorders, Panic disorders, Panic Attacks, Generalized Anxiety Disorder, or any associated DSM diagnosis or condition. 18. Subject has a history of or currently has hypoventilation syndrome or sleep apnea and is on non-invasive ventilation. 19. Subject has a history of unexplained syncope, structural heart disease, long QT syndrome, heart failure, myocardial infarction, angina, unexplained cardiac arrhythmia, TdP, ventricular tachycardia, or placement of a pacemaker or implantable defibrillator. Subjects will be also excluded if there is a family history of long QT syndrome (genetically proven or suggested by sudden death of a close relative to cardiac causes at a young age) or Brugada syndrome. 20. Subject has a history of suicidal ideation or previous suicide attempts. 21. Subject has a safety 12-lead ECG result at screening or check-in at any study period with evidence of any of the following abnormalities: • QTc using Fridericia correction (QTcF)>430 msec • PR interval>220 msec or <120msec • QRS duration>110 msec • Second- or third-degree atrioventricular block • Complete left or right bundle branch block or incomplete right bundle branch block • Heart rate <50 or >90 beats per minute • Pathological Q-waves (defined as Q-wave>40 msec) • Ventricular pre-excitation

	22. Subject has a skin condition likely to compromise ECG electrode placement. 23. Any individual with breast implants.
Sample Collection	The following samples should be collected and processed as follows. Number of samples collected throughout the study can be found in the Schedule of Events (see Appendix): The PK blood samples (6 mL each) will be collected into tubes containing K2EDTA, inverted several times to mix the blood with the anticoagulant, and placed in an ice bath. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes, at 3000 relative centrifugal force (RCF), at 4°C, by a study team member. The plasma will be separated using a disposable plastic pipette and equally aliquoted into duplicate cryotube vials labeled as Aliquot A (primary) and Aliquot B (backup). The plasma samples will be appropriately labeled and stored frozen at -70°C or below within 30 minutes after aliquoting until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor. The Aliquot A samples (primary) will be shipped first, on dry ice, to the bioanalytical laboratory at FDA for processing when requested by the sponsor. The Aliquot B samples (backup) will be held for a second shipment after completion of all Aliquot A sample shipment(s) and the timing of the Aliquot B shipment will be communicated by the sponsor. None of the PK blood samples will be stored at the clinical facility for future use.
Pharmacokinetic Assessments:	Pharmacokinetic blood samples will be collected on the following days at the approximate times for each study period: • Day 5, 6, 11, 12, 20, and 21: 0 (pre-dose), 3, 4, 5, 8 hr (and 24 hr for Day 6, 12, and 21) • Day 2 and 16: 0 (pre-dose), 2, 3, 4, 5, 6, 8, 12, 24 hr Blood samples will be collected by direct venipuncture or by inserting an IV catheter. There will be a total of 51 PK samples per participant for each study period. For each participant, the following PK parameters will be determined for oxycodone and oxymorphone (days 6, 12, and 21) and paroxetine escitalopram, and escitalopram metabolites (days 2, 5, 6, 11, 12, 16, 20, and 21): • Maximum observed plasma concentration (C_{max}) • Area under the plasma concentration time curve (AUC)

	• Time at which C_{max} occurs (T_{max}) • Elimination rate constant (K_{el}) • Terminal half-life ($t_{1/2}$) • Accumulation ratio (for escitalopram and paroxetine)
Pharmacodynamic Assessments:	Rebreathing assessments following Duffin's rebreathing procedure (see separate Standard Operating Procedure [SOP]) will be performed on the following study days and time of each study period: • Day -1: one hyperoxic and one hypoxic assessment • Day 5, 6, 11, 12, 20, and 21: hyperoxic assessments at 0 (pre-dose), 4 and 5 hours; one hypoxic assessment at 5 hours (following the hyperoxic assessment) During Duffin's rebreathing procedure, subjects will be placed in an upright position (90^o angle) on a bed and connected to the respiration measurement set up. Subjects will wear a facemask and breathe from a pre-filled rebreathing bag containing approximately 4 L of either a hyperoxic-hypercapnic (24% O_2, 6% CO_2, N_2 balanced) or hypoxic-hypercapnic (6% O_2, 6% CO_2, N_2 balanced) gas mixture. During this study, subjects will be asked to breath room air for 5 minutes (relaxation stage) and then to hyperventilate (primarily through deep breathing) room air voluntarily for 5 minutes (hyperventilation stage). Thereafter, subjects will be switched to the rebreathing bag and perform rebreathing assessments at one of the 2 different isoxic end tidal PO_2, i.e., 150 mmHg and 50 mmHg. The isoxia at 150 mmHg or 50 mmHg end-tidal PO_2 will be maintained by providing a computer-controlled flow of 100% O_2 to the rebreathing bag. Pupillary assessments (see separate SOP) will be performed before and after each rebreathing procedure (except not in between the hyperoxic and hypoxic paired assessment) while the ambient light is kept at a consistent level. The rubber cup of the pupillometer will be placed on the right eye and repeated on the same eye approximately 1 minute later. ECG assessments will be performed on the following study days and time of each study period: • Day 1: 0 (pre-dose) hr • Day 2 and 16: 0 (pre-dose), 2, 3, 4, 5, 6, 8, 12, 24 hr Ten second 12-lead ECG tracings with the most stable heart rate and highest signal-to-noise ratio will be extracted in at least 3 replicates at ECG extraction time points during each treatment period. During all ECG extraction periods, subjects will be placed in a supine position. Three ECG extractions will be performed for a baseline

	measurement before the first oral dose on day 1 of each treatment period. The other ECG extractions/period time matched to the PK samples will be obtained before the PK sample collection time. Subjects will be isolated (not able to see each other) in a quiet environment that is free from external stimuli. Different ventilation, cardiovascular, pupillary, sedation, and ECG measures that will be summarized include, but are not limited to, the following: **Ventilation and Cardiovascular Assessments:** • During rebreathing stage o *VE55* (minute ventilation at the 55mm Hg end tidal CO_2 point) o *Baseline minute ventilation* when end tidal PCO_2 is less than the ventilatory recruitment threshold (represents non-chemoreflex drives to breathe) o *Ventilatory recruitment threshold* (end tidal PCO_2 above which minute ventilation starts to increase linearly with further increases in end tidal PCO_2) o *Slope of the minute ventilation / end-tidal partial pressure of carbon dioxide (end-tidal PCO_2) regression line* that reflects the increase in minute ventilation relative to the increase in end tidal PCO_2 (represents chemoreflex sensitivity) o *Extrapolated ventilation recruitment threshold* (intersection with X axis) • During relaxation stage o Minute ventilation, tidal volume, respiratory rate, end-tidal PCO_2, end-tidal PO_2, oxygen saturation, and heart rate during the relaxation stage o Number of apneic events lasting > 10 s during the relaxation stage **Pupillary Assessments** • Maximum pupil diameter before constriction • Minimum diameter at peak constriction • Percent change between min/max diameter • Latency of constriction • Average constriction velocity • Maximum constriction velocity • Dilation velocity after peak constriction • Time to reach 75% recovery of maximum diameter

	Sedation scores • Ramsey Sedation Scale • Visual Analogue Scale **ECG Assessments** • Change from baseline in QTc, PR, QRS, JT_{peakC}, $T_{peak}T_{end}$ and heart rate. In addition, PK/PD relationships will be evaluated between study drugs and a subset of pharmacodynamic endpoints (ventilation, pupillary, ECG).
Safety Assessments:	Safety will be evaluated in terms of adverse events (AEs), clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG, and physical examination findings.
Sample Size and Threshold Determination:	Approximately 25 healthy participants are planned for enrollment. A previous study (20 participants) with paroxetine showed a decrease of 10.2 L/min in VE55 for paroxetine (40 mg) combined with oxycodone (10 mg) compared to oxycodone alone after 5 days of dosing with paroxetine. Similarly, there was a 9.3 L/min decrease in VE55 for paroxetine compared to placebo after 4 days of dosing with paroxetine. The standard deviation in assessments was approximately 6.5 L/min. Assuming a similar effect size with paroxetine and escitalopram at day 20 and 21, the planned number of participants allows for a 40% drop out rate while maintaining at least 90% power for separate one-sided tests at a 0.025 significance level with the multiple primary endpoints.
Statistical Methods:	All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics. The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized. Demographic and baseline characteristics will be summarized overall and by treatment for all subjects. Descriptive statistics will be used to summarize demographic and baseline subject characteristics. For continuous variables, the mean, median, standard deviation (SD), minimum, and maximum values will

	be reported. For categorical (nominal) variables, the number and percentage of subjects (or observations) will be reported. The rebreathing analysis population will include all subjects who completed at least one rebreathing assessment on any day or at any timepoint. Specific information on analysis populations for different pharmacodynamic measures will be described in the Statistical Analysis Plan (SAP). **<u>Primary and Secondary Ventilation Analyses</u>** Multiple primary analyses are planned for each drug. The first primary analysis will be VE55 on day 21 at 5 h (hyperoxic) between oxycodone with paroxetine or escitalopram compared to oxycodone alone. The second primary analysis will be VE55 on day 20 at 5 h (hyperoxic) between paroxetine or escitalopram compared to placebo. Since Day 20 and 21 are considered multiple primary endpoints, comparisons will have an adjustment for multiplicity using a Bonferroni correction (each day will be tested at a 0.025 significance level). No adjustments will be made for separate testing of paroxetine or escitalopram since the treatments are considered separate interventions in the study. A linear mixed effects model will be developed using all study data from hyperoxic assessments. Fixed effects will include treatment, period, sequence, and baseline VE55 (i.e., VE55 obtained at 0 h on Day -1 of each treatment period). Subject will be included as a random effect on the intercept. If a subject's baseline VE55 for a treatment period is not available, then the baseline value for that period will be calculated based on the median of all other baseline values for that subject from other study periods. If a subject does not have any completed baseline VE55 data, the subject will be assigned a baseline VE55 equal to the median of the population. To demonstrate an effect when oxycodone is combined with paroxetine (or escitalopram) compared to oxycodone the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments must not overlap 0 L/min. Similarly, to demonstrate an effect of paroxetine (or escitalopram) compared to placebo the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments must not overlap 0 L/min. Each comparison will be performed separately (i.e.,'trt' as 'paroxetine' or 'escitalopram').: • H0: VE55oxy+trt - VE55oxy ≥ 0 L/min • HA: VE55oxy+trt - VE55oxy < 0 L/min

	For secondary endpoints, effects of paroxetine or escitalopram with oxycodone compared to oxycodone alone will also be performed using the same comparisons on day 6 and day 12. As additional secondary endpoints, effects of paroxetine or escitalopram alone compared to placebo will be determined on day 5 and 11 using the same model. Additional details on testing of ventilation secondary endpoints are discussed in the SAP. **Pharmacokinetics**: Concentrations will be determined at approximately specified timepoints. Oxycodone and oxymorphone C_{max} and AUC will be summarized on days 6, 12, and 21 using descriptive statistics. Differences in oxycodone or oxymorphone alone compared to exposures when coadministered with paroxetine or escitalopram will be compared. C_{max} and AUC will be log-transformed and the values between study arms will be compared using a linear mixed effects model with treatment as a categorical variable and participant as a random effect. Paroxetine and escitalopram C_{max} and AUC will be summarized on days 2, 5, 6, 11, 12, 16, 20 and 21 using descriptive statistics. The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using SAS or R software. Mean and individual concentration time profiles will be presented in graphs. Additional PK parameter analyses including but not limited to measures listed under Pharmacokinetic Assessments will be included in the SAP. **Additional Pharmacodynamics on Ventilatory Assessment Days**: Mean and standard deviations for baseline minute ventilation, ventilatory recruitment threshold, slope of the PCO_2-ventilatory response curve, and the extrapolated ventilatory recruitment threshold will be calculated for each subject at each rebreathing assessment using data from the rebreathing stage. Mean and standard deviation for minute ventilation, tidal volume, respiratory rate, end-tidal PCO_2, end-tidal PO_2, oxygen saturation, and heart rate will be calculated using data from the relaxation stage. Number of apneic events lasting > 10s will be counted for each subject, day, and rebreathing assessment during the relaxation stage. Full details will be described in a separate SAP. Additional pupillary assessments (e.g., ventilation, respiratory rate, heart rate, maximum pupil diameter before constriction) as specified in the protocol will be reported with standard descriptive statistics. **ECG Assessments**

	Intensive ECG assessments will be performed on Days 2 and Day 16. The QT interval will be corrected for heart rate using Fridericia's formula ($QTc=QT/RR^{1/3}$). The J-T_{peak} interval will be corrected for heart rate using the formula ($J\text{-}T_{peakC} = J\text{-}T_{peak}/RR^{0.58}$). Baseline will be the mean of the 3 predose ECG extractions of Day 1. Exposure-response analyses will be performed for the change from baseline in QTc (and other ECG measurements; see ECG Analysis Plan), where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline. The concentration of the drug will be used as a covariate. Exposure-response analysis will be done following most recent best practices in concentration-QTc modeling. Additional details will be specified in the ECG Analysis Plan. **Safety** The safety population will include all subjects who receive at least 1 dose of the study drug. All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs, organized by system organ class and preferred term, will be summarized with a focus on treatment-emergent AEs. Vital sign measurements will be summarized using descriptive statistics by time point. All values will be evaluated for clinically notable results. Data for additional safety parameters (e.g., physical examination findings) will be listed.
Date of Protocol:	21 July 2022

1. INTRODUCTION

A well-known and potentially fatal adverse reaction associated with opioid administration, particularly in the scenario of misuse, abuse, or when co-administered with certain other drugs is that people 'stop breathing'. Research suggests this is caused by a reduced respiratory response to counteract increasing levels of systemic carbon dioxide (CO_2). In August 2016, the U.S. Food and Drug Administration (FDA) included boxed warnings about increased potential for respiratory depression with co-use of benzodiazepines and opioids.[1] In response to this action, concerns were raised because patients may be prescribed other psychotropic drugs that may have similar adverse reactions when combined with opioids.

Subsequently, FDA performed a review of the literature and identified 13 psychotropic drugs for evaluation in an in vivo animal study (using a rat model). The in vivo study identified 4 drugs that had significant effects when combined with oxycodone on arterial carbon dioxide.[2] Two drugs (quetiapine and paroxetine) were selected for further evaluation in a human clinical study evaluating drug effects on hypercapnic ventilation.

To study the effects of opioids alone or in combination with other psychotropic drugs on ventilation, FDA used a procedure referred to as Read rebreathing.[3-4] With Read rebreathing, study participants rebreathe through a circuit with increased level of O_2 and CO_2 (93% O_2 and 7% CO_2). The artificially increased levels of CO_2 trigger the subjects to increase ventilation. This "hypercapnic ventilatory response" can be decreased by opioids, benzodiazepines, and other drugs.[5-19]

The previous study, which included 5-days of study drug dosing with oxycodone administered on day 1 or 5 showed quetiapine co-administered with oxycodone did not cause a significant decrease in hypercapnic ventilation compared to oxycodone alone.[20] In contrast, paroxetine caused a decrease in hypercapnic ventilation compared to oxycodone alone, and an additive effect was seen when combined with oxycodone. This mechanism is due to a direct pharmacodynamic effect, rather than a pharmacokinetic interaction and most likely due to its serotonergic properties. Paroxetine is highly selective for inhibiting the serotonin transporter versus other monoamine transporters and other receptors in general.[21-22] Although different types of serotonergic receptors and neurons can have differential effects on breathing, paroxetine does not bind to serotonin receptors at clinically-relevant exposures.[23-24] In addition, nonclinical and clinical studies have shown different selective serotonin reuptake inhibitors (SSRIs) can have independent effects on ventilation, though SSRIs have not been shown to cause severe respiratory depression on their own.[25-43] This supports the need for additional investigation on the effects of serotonergic drugs. Escitalopram is highly selective for the serotonin transporter, has low selectivity for serotonin receptors and muscarinic

receptors, and is widely prescribed, making it a good candidate as a second SSRI to evaluate in this study.

The previous study evaluated effects on ventilation over 5-days of dosing. SSRIs take approximately 3 weeks to reach maximal therapeutic effect, which correlates with the time required for pre-synaptic inhibitory serotonergic receptors to desensitize.[44-46] SSRI effects on ventilation in the proposed study would be evaluated for a similar period of time to confirm if effects on ventilation persist under steady state conditions.

Finally, an additional modification in the proposed study will be the assessment of hypercapnic ventilation under hyperoxic and hypoxic gas levels using a modified Read rebreathing approach referred to as Duffin rebreathing. One limitation of the rebreathing procedure from the previous study design is all measurements are performed under hyperoxic conditions, which can have independent effects on ventilation. In addition, the data cannot necessarily be used to extrapolate to the dynamic changes in PO_2 and PCO_2 that occur during real-world drug-induced respiratory depression when a patient is breathing room air. Duffin modified the Read rebreathing method to include 1) hyperventilation (primarily through deep breathing) prior to rebreathing to achieve an end tidal PCO_2 or approximately 20-25 mm Hg prior to rebreathing, 2) maintaining an isoxic end tidal PO_2 during rebreathing, and 3) repeating rebreathing at 2 different isoxic end tidal PO_2 levels (i.e., at 150 mm Hg and 50 mm Hg). These modifications, which have been used by investigators for decades, allow for critical physiological measurements and thresholds to be captured that can then be used to model the effects of drugs when there are dynamic changes in PO_2 and PCO_2, such as during a real-world opioid overdose.[47-52]

2. STUDY OBJECTIVES

2.1 Primary Objectives

- To study ventilatory effects of SSRIs (paroxetine or escitalopram) combined with oxycodone compared to oxycodone alone after 21 days of SSRI dosing.
- To study ventilatory effects of SSRIs (paroxetine or escitalopram) compared to placebo after 20 days of SSRI dosing.

2.2 Secondary Objectives

- To compare ventilatory effects of SSRIs (paroxetine or escitalopram) combined with oxycodone compared to oxycodone alone after 6 and 12 days of SSRI dosing.

- To compare ventilatory effects of SSRIs (paroxetine or escitalopram) compared to placebo after 5 and 11 days of SSRI dosing.

2.3 Exploratory Objectives

- To study whether paroxetine or escitalopram affects the pharmacokinetics of oxycodone.
- To evaluate differences in hypercapnic ventilatory response under hyperoxic or hypoxic rebreathing.
- To study whether there is a direct pharmacodynamic interaction between paroxetine or escitalopram and oxycodone.
- To summarize additional pharmacokinetic parameters and pharmacodynamic measurements collected during the study.

3. STUDY ENDPOINTS

3.1 Primary Endpoints

The following primary endpoints will be evaluated:

- Minute ventilation at 55mm Hg end tidal CO_2 (VE55) under hyperoxic conditions on day 21
- VE55 under hyperoxic conditions on day 20

3.2 Secondary Endpoints

The following secondary endpoints will be evaluated:

- VE55 under hyperoxic conditions on days 6 and 12
- VE55 under hyperoxic conditions on days 5 and 11

3.3 Exploratory Endpoints

The following exploratory PK parameters will be determined for oxycodone and oxymorphone (days 6, 12, and 21) and paroxetine, escitalopram, and escitalopram metabolites (days 2, 5, 6, 11, 12, 16, 20, and 21):

- Maximum observed plasma concentration (C_{max})
- Area under the plasma concentration time curve (AUC)
- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})

- Terminal half-life ($t_{1/2}$)
- Accumulation ratio (for escitalopram and paroxetine)

Additionally, the following exploratory PD markers may be evaluated:

Ventilation and Cardiovascular Assessments:

- During rebreathing stage
  - *VE55* (minute ventilation at the 55mm Hg end tidal CO_2 point)
  - *Baseline minute ventilation* when end tidal PCO_2 is less than the ventilatory recruitment threshold (represents non-chemoreflex drives to breathe)
  - *Ventilatory recruitment threshold* (end tidal PCO_2 above which minute ventilation starts to increase linearly with further increases in end tidal PCO_2)
  - *Slope of the minute ventilation / end-tidal partial pressure of carbon dioxide (end-tidal PCO_2) regression line* that reflects the increase in minute ventilation relative to the increase in end tidal PCO_2 (represents chemoreflex sensitivity)
  - *Extrapolated ventilation recruitment threshold* (intersection with X axis)
- During relaxation stage
  - Minute ventilation, tidal volume, respiratory rate, end-tidal PCO_2, end-tidal PO_2, oxygen saturation, and heart rate during the relaxation stage
  - Number of apneic events lasting > 10 s during the relaxation stage

Pupillary Assessments

- Maximum pupil diameter before constriction
- Minimum diameter at peak constriction
- Percent change between min/max diameter
- Latency of constriction
- Average constriction velocity
- Maximum constriction velocity
- Dilation velocity after peak constriction
- Time to reach 75% recovery of maximum diameter

Sedation scores

- Ramsey Sedation Scale

- Visual Analogue Scale

ECG Assessments

- Change from baseline in QTc, PR, QRS, J-T_{peakC}, T_{peak}-T_{end} and heart rate.

An exploratory endpoint is the pharmacokinetic/pharmacodynamic (PK/PD) relationship for study drugs when administered alone versus in combination.

4. INVESTIGATIONAL PLAN

4.1 Study Design

This study will be a randomized, double blind, three period crossover study with approximately 25 healthy volunteer participants. This study will include three 21-day treatment periods with a 3-week washout between each period. Each subject will be randomized to 1 of 6 treatment sequences (i.e., ABC, ACB, BAC, BCA, CAB, CBA). FDA will prepare the randomization schedule.

Table 4-1: Study Schedule

Day -1	Days 1-21	Days 22-42	Days 42-63	Days 64-84	Days 85-105	Day 106
Check-in	Period 1	Washout	Period 2	Washout	Period 3	Check-out

Table 4-2: Study Treatments:

Treatment	Day					
	1-5	6	7-11	12	13-20	21
A	Placebo	Placebo + 10 mg oxycodone	Placebo	Placebo + 10 mg oxycodone	Placebo	Placebo + 10 mg oxycodone
B	40 mg paroxetine	40 mg paroxetine + 10 mg oxycodone	60 mg paroxetine	60 mg paroxetine + 10 mg oxycodone	60 mg paroxetine	60 mg paroxetine + 10 mg oxycodone
C	20 mg escitalopram	20 mg escitalopram + 10 mg oxycodone	30 mg escitalopram	30 mg escitalopram + 10 mg oxycodone	30 mg escitalopram	30 mg escitalopram + 10 mg oxycodone

Participants will enter the clinic on a staggered basis in cohorts of approximately five so that no more than five subjects are undergoing rebreathing on any given day. Subjects will be staggered to allow for direct safety overview by medical staff during Duffin's rebreathing.

Participants will receive either placebo, paroxetine, or escitalopram on days 1-21 for each period. Oxycodone will be administered on days 6, 12, and 21 of each period. Dosing of the study drugs will occur at time 0 on each day. Oxycodone dosing will occur at 3 h so

that maximum concentration occurs for all drugs is at approximately the same time. Participants will also receive ondansetron 4 mg 30 min before oxycodone administration.

Participants will be confined to the study clinic from Day -1 the morning of Day 22 for each period. There will be a three-week washout between each period. Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days.

Upon return to clinic, on Days 42 & 84, eligibility criteria to continue study participation will be reviewed (see Schedule of Events for criteria listed on Days 42 & 84), any changes in medical history (including concomitant medications) will be documented.

4.1.1 Risk/Benefit

Subjects will be informed that participation in a human clinical pharmacology study like the present one cannot be of benefit to healthy volunteers. Nevertheless, the information from the physical examination, vital sign measurements, and ECG results may be shared with the subject's personal physician if this is the subject's choice. Subjects will be informed that it is also their choice to inform their personal physician that they are participating in this research study.

Subjects will be informed that their contribution to the study is of major importance to agencies like the FDA to understand the potential for respiratory depression with coadministration of SSRIs and opioids. However, since this is a study involving healthy volunteers, subjects will be informed that they have the option not to participate.

Subjects will be informed that opioids have a box warning for life-threatening respiratory depression. To mitigate potential events, subjects will undergo continuous pulse oximetry monitoring, there will be stopping rules for the treatment, rescue medications for oxycodone will be present, and a safety management plan for staff will be implemented during the study which includes a requirement to have 1:1 advanced cardiac life support staff to subjects in addition to the study investigator.

Subjects will be informed that they may be exposed to risks associated with the pharmacological properties of the study drug and the study procedures. The following summary of potential AEs for the study drugs will be provided to and discussed with the subjects:

1. Oral oxycodone 5 mg IR tablets (up to 10 mg): The most common adverse events include typical opioid-related adverse reactions: nausea, constipation, vomiting, headache, pruritus, insomnia, dizziness, asthenia, agitation, anxiety, hallucinations, nightmares, and somnolence. Serious adverse reactions include: respiratory

depression, respiratory arrest, circulatory depression, cardiac arrest, hypotension, and/or shock.[53]

2. Oral paroxetine 20 mg IR tablets (up to 60 mg): The most common adverse events include: somnolence, insomnia, agitation, tremor, anxiety, dizziness, constipation, nausea, diarrhea, dry mouth, vomiting, flatulence, asthenia, erectile dysfunction, delayed ejaculation, vaginal paresthesia, itching, and discharge, yawning, infection, sweating, decreased appetite, nervousness, impotence, and libido decreased.[54]
3. Oral escitalopram 10 mg tablets (up to 30 mg): The most common adverse events include: insomnia, ejaculation disorder, drowsiness, impotence, nausea, sweating increased, fatigue, somnolence, decreased libido, anorgasmia, headache, diarrhea, dry mouth, dizziness, constipation, and indigestion. Additional adverse events include hypersensitivity reactions, seizures, syncope, QT interval prolongation, cardiac arrhythmias, enhanced clotting, and hepatic enzyme elevation.[55]
4. Oral ondansetron 4 mg tablets given prior to oxycodone for nausea/vomiting and 4 mg IV as needed: The most common adverse events include: diarrhea, headache, fever, malaise, fatigue, constipation, and hypoxia. Additional adverse events include: hypersensitivity reactions, including anaphylaxis and bronchospasm, QT interval prolongation and Torsade de Pointes, and serotonin syndrome (usually when prescribed with another serotoninergic medication such as select serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors).[56]

Selected doses of individual drugs are within the FDA approved labeled doses or are doses that have been used in prior healthy participant studies for similar durations. As drug combinations are being studied, the doses of oxycodone are less than that which has been used in prior studies in opioid naïve healthy volunteers undergoing rebreathing[5-6,11] or other similar procedures (see below).

- Oxycodone - A prior study of the effects of decreased ventilatory response to hypercapnia of oxycodone with and without ethanol in opioid naïve healthy volunteers (young and elderly participants) used a dose of oxycodone 20 mg.[5-6] An additional ongoing study from these authors (unpublished) is using a dose of oxycodone 40 mg in opioid naïve healthy volunteers. Our previous study (unpublished) used 10 mg oxycodone, which decreased ventilation approximately 5 L/min from baseline and was well-tolerated by study participants with no discontinuations due to the selected dose. The present study will also use oxycodone 10 mg.
- Paroxetine – Paroxetine will be administered 40 mg/day for 6 days and 60 mg/day for 15 days. This is within the maximum labeled dose of paroxetine (60 mg/day). The dose is higher than the typical starting dose of 20 mg/day that is titrated up

over 1-week intervals, however starting doses up to 60 mg have been given safely to healthy volunteers.[51]

- Escitalopram – Escitalopram will be administered at 20 mg/day for 6 days and 30 mg/day for 15 days. This is above the maximal labeled dose of escitalopram (20 mg/day) but doses up to 30 mg/day have been evaluated in a thorough QT study. The exposure with 30 mg is similar to the steady state concentration expected in CYP2C19 poor metabolizers following a therapeutic dose of 20 mg.[55]
- Ondansetron – Ondansetron will be administered as a single 4 mg oral dose in all treatment periods prior to administration of oxycodone and will be available as 4 mg IV as needed for nausea and vomiting. A dose of 4 mg is less than the highest labeled dosing for other ondansetron indications (24 mg as a single dose or 8 mg twice a day).[56]

The study drugs will not be administered to anyone who is pregnant. All women must take a pregnancy test before receiving any study drug in this study. All women of childbearing potential enrolled on this study will be informed that they must use effective birth control methods (abstinence, intrauterine device, and contraceptive foam and a condom [i.e., double-barrier method]) during treatment. Subjects will be informed that they must notify the investigator if they or their female partners become pregnant during the course of the study. If a subject becomes pregnant, she will be informed that neither Spaulding Clinical Research nor the sponsor will be responsible for the cost of any obstetric or related care, or for the child's care.

Subjects will be informed that insertion of an IV catheter may be required for blood sample collection and, during insertion of the catheter, soreness, bruising, or infection at the insertion site are possible but unlikely. Subjects will also be informed that dizziness and lightheadedness may occur during direct venipuncture, insertion of the IV catheter, or during blood collection.

Subjects will be informed that they will be participating in the Duffin rebreathing procedure where they will breathe a hyperoxic-hypercapnic (24% O_2, 6% CO_2, N_2 balanced) or hypoxic-hypercapnic (6% O_2, 6% CO_2, N_2 balanced) gas mixture through a mask. They will be informed that this mixture of oxygen, carbon dioxide and nitrogen provided will be different than is normally found in room air (21% O_2, 0.04% CO_2, N_2 balanced with minor amounts of other gasses). Subjects will be informed that the different gas concentrations, in particular the higher level of carbon dioxide breathed in, may result in a feeling of needing to breathe faster or more deeply. Subjects will be informed that this procedure has been used in many previous physiologic measurement studies and is non-invasive. During screening, subjects will be shown the rebreathing equipment and will be trained how to use it to understand if the procedure is tolerable.

Subjects will be informed that the confidentiality of their data will be respected at all times according to state law, and the study personnel handling their study data are bound by confidentiality agreements.

Subjects will be informed that extra precautions will be put in place, including required screening tests, that will limit the risk of COVID-19. Precautions will be documented in a COVID-19 risk management plan. Subjects will be informed that despite the extra precautions there is still a risk of them contracting COVID-19. Any changes to the COVID-19 precautions (e.g., due to updated CDC recommendations or new testing becoming available) will be documented in the COVID-19 risk mitigation plan.

Subjects will be informed that the study drug and all tests, procedures, and visits required by the study are provided at no cost to them. If subjects become ill or physically injured because of participation in this study, they will be informed that costs of treatment will not be covered by the sponsor.

4.2 Selection of Study Population

Subjects will be screened, and the data collected will be reviewed by the principal investigator. Only those subjects who meet all the eligibility criteria will be enrolled. Approximately 25 healthy subjects are planned for enrollment. Every effort will be made to maintain an approximate 50:50 male-to-female sex distribution.

4.2.1 Inclusion Criteria

Subjects who meet all the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an IRB-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization) before any study-related procedures are performed.
2. Subject is a healthy, non-smoking man or woman, 18 to 50 years of age, inclusive, who has a body mass index of 18.5 to 33.0 kg/m^2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, pulse oximetry, 12-lead ECG results, and physical examination findings at screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).
4. Subject must have a negative test result for alcohol and drugs of abuse at screening and check-in days.
5. Subject must test negative for severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) by a rapid antigen test at check-in for all study periods. If a subject's test comes back as invalid, the test can be repeated.

6. Female subjects must be of non-childbearing potential (confirmed with follicle-stimulating hormone levels > 40 mIU/mL) or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before check-in (Day -1) and agree to remain strictly abstinent for the duration of the study and for at least 1month after the last application of study drug; OR 2) be practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before check-in (Day -1) until at least 1 month after the end of the study.
7. Male subjects must agree to practice 2 highly effective methods of birth control (as determined by the investigator or designee) from at least 1 month before check-in (Day -1) until at least 1 month after the last dose of study drug.
8. Subject is highly likely (as determined by the investigator) to comply with the protocol defined procedures and to complete the study.

4.2.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject has used any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer) or complementary and alternative medicines within 28 days before the first dose of study drug. This includes prescription or nonprescription ophthalmic drugs.
2. Subject is currently participating in another clinical study of an investigational drug or has been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.
3. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff, electronic cigarettes) within 6 weeks of Screening. Subjects must refrain from using these throughout the study.
4. Subject has consumed alcohol, xanthine containing products (e.g., tea, coffee, cola), caffeine, grapefruit, or grapefruit juice within 24 h of check-in. Subjects must refrain from ingesting these throughout the study.
5. Subject has a history or evidence of a clinically significant disorder, condition, or disease (e.g., cancer, human immunodeficiency virus [HIV], hepatic or renal impairment) that, in the opinion of the investigator would pose a risk to subject safety or interfere with the study evaluation, procedures, or completion. This includes subjects with any underlying medical conditions that put subjects at

increased risk of severe illness from COVID-19 based on the Centers for Disease Control and Prevention (CDC) guidelines.

6. Subject has any signs or symptoms at screening or check-in of any study periods that are consistent with COVID-19. Per current CDC recommendations this includes subjects with the symptoms cough or shortness of breath or difficulty breathing, or at least two of the following symptoms: fever, chills, repeated shaking with chills, muscle pain, headache, sore throat or new loss of taste/smell. In addition, the subject has any other findings suggestive of COVID-19 risk in the opinion of the investigator.
7. Subject has known or suspected allergies or sensitivities to any study drugs.
8. Subject has clinical laboratory test results (hematology, serum chemistry and urinalysis) at Screening or period check-in that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator. Clinical laboratory results may be repeated once, as needed, for confirming results at Screening and period check-in.
9. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.
10. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.
11. Female subject is currently pregnant or lactating or was within 3 months of enrollment.
12. Subject has a history of opioid or psychotropic drugs within 60 days of the start of the study.
13. Subject has a history of asthma that has required medication within the last five years.
14. Subject has non-reactive or mishappen pupil(s) or damaged orbit structure or surrounding soft tissue is edematous or has an open lesion.
15. Subject has a Mallampati score of >2.
16. Subject's Duffin rebreathing data is of poor quality or subject does not agree to remain clean-shaven for all days when the Duffin rebreathing procedure is performed.
17. Subject has a history of sleep disorders, Panic disorders, Panic Attacks, Generalized Anxiety Disorder, or any associated DSM diagnosis or condition.

18. Subject has a history of or currently has hypoventilation syndrome or sleep apnea and is on non-invasive ventilation.
19. Subject has a history of unexplained syncope, structural heart disease, long QT syndrome, heart failure, myocardial infarction, angina, unexplained cardiac arrhythmia, TdP, ventricular tachycardia, or placement of a pacemaker or implantable defibrillator. Subjects will be also excluded if there is a family history of long QT syndrome (genetically proven or suggested by sudden death of a close relative to cardiac causes at a young age) or Brugada syndrome.
20. Subject has a history of suicidal ideation or previous suicide attempts.
21. Subject has a safety 12-lead ECG result at Screening or check-in at any study period with evidence of any of the following abnormalities:
    - QTc using Fridericia correction (QTcF)>430 msec
    - PR interval>220 msec or <120msec
    - QRS duration>110 msec
    - Second- or third-degree atrioventricular block
    - Complete left or right bundle branch block or incomplete right bundle branch block
    - Heart rate <50 or >90 beats per minute
    - Pathological Q-waves (defined as Q-wave>40 msec)
    - Ventricular pre-excitation
22. Subject has a skin condition likely to compromise ECG electrode placement.
23. Any individual with breast implants.

4.3 Screening Failures

Subjects who sign and date the informed consent form but who fail to meet the inclusion and exclusion criteria are defined as screening failures. A screening log, which documents the subject initials and reason(s) for screening failure, will be maintained by the investigator for all screening failures. A copy of the log should be retained in the investigator's study files.

If a subject fails the screening process because of an abnormal laboratory result, they can receive a copy of the results upon request. The investigator will determine if follow-up for the abnormal laboratory result is needed and will encourage the subject to follow-up with his or her personal physician as appropriate. All subjects will be informed as to the

reason(s) they are excluded from study participation, even if follow-up is not required. If a subject fails the screening process because of a positive test result for human immunodeficiency virus or hepatitis, the positive result will be reported to local health authorities as required by law.

4.4 Termination of Study or Investigational Site

4.4.1 Criteria for Termination of the Study

The study will be completed as planned unless one of the following criteria is satisfied that requires early termination of the study.

- New information regarding the safety or efficacy of the study drug(s) that indicates a change in the known risk profile for the study drug(s), such that the risk is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objective or compromises subject safety.

4.4.2 Criteria for Termination of the Investigational Site

The study site may be terminated if the site (including the investigator) is found in significant violation of GCP, the protocol, the contractual agreement, or is unable to ensure adequate performance of the study.

In the event that the sponsor elects to terminate the study or the investigational site, a study-specific procedure for early termination will be provided by the sponsor; the procedure will be followed by the applicable investigational site during the course of termination.

4.5 Criteria for Subject Withdrawal

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn by the investigator without the approval of the subject based on the investigator's clinical judgment. A subject is not required to provide a written request to withdraw from the study; however, a written request is required if a subject withdraws consent for his or her personal data to be used for study-related purposes.

A subject may be discontinued for any of the following reasons:

- AE: The subject has experienced an AE that, in the opinion of the investigator, requires early termination. The appropriate electronic case report form (eCRF) must be completed for each AE. If a subject is discontinued from the study due to an AE, the investigator is required to follow-up with the subject until the event resolves or becomes stable. If a subject dies during the study, the cause of death must be reported as a serious AE (SAE), with an outcome of death noted in the eCRF.

- Protocol Violation: The subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unnecessary risk to the subject's health.
- Withdrawal by Subject: The subject (or other responsible individual [e.g., caregiver]) wishes to withdraw from the study in the absence of a medical need.

  NOTE: Withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category.
- Study Terminated by Sponsor: The sponsor, IRB, FDA, or other regulatory agency terminates the study.
- Pregnancy: The subject is found to be pregnant.

  NOTE: If the subject is found to be pregnant, the subject must be withdrawn immediately. The pregnancy will be followed-up to term, and the outcome, including any premature termination will be recorded. All live births must be followed for a minimum of 30 days or until the first well-baby visit.
- Other.

  NOTE: This category records withdrawals caused by an accidental or a medical emergency, unblinding, and other rare cases. The specific reason should be recorded in the comment space of the eCRF.

4.5.1 Handling of Withdrawals

The investigator may terminate a subject's study participation at any time during the study when the subject meets the criteria described in Section 4.5. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Subjects will be informed that their participation in the study is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Should a subject's participation be discontinued, the primary reason for termination must be recorded. In addition, efforts should be made to perform all procedures scheduled for the early termination visit. Any data and samples collected before subject withdrawal will become the property of the sponsor.

4.5.2 Replacement of Subjects

Approximately 25 healthy subjects are planned for enrollment and will be randomized to 1 of 6 treatment sequences.

4.6 Study Visits

4.6.1 Recruitment

Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local IRB before telephone screening. The sponsor is responsible for registration of the study on clinicaltrials.gov. Recruitment may not occur until the study is fully registered on clinicaltrials.gov.

4.6.2 Compensation

Subjects will be offered payment for Screening; however, if the results of their alcohol and drug screening tests are positive, they will not be compensated. Subjects who complete the entire study will receive payment according to the schedule provided in the informed consent form. No special incentives are offered. Final payment will not be released until all follow-up procedures have been completed and accepted by the investigator.

If a subject chooses to withdraw from the study prematurely, he or she will only be compensated for completed days. If subjects are withdrawn for medical reasons or if the study is halted temporarily or permanently, the subjects will receive compensation proportional to the time spent in the study. No compensation will be provided if a subject is dismissed from the study for noncompliance (e.g., improper conduct, ingesting alcohol and/or drugs [including recreational drugs], tampering with the study drug, consuming any prohibited foods or beverages).

If subjects are required to stay in the clinic for a longer period for safety reasons, they will be compensated at a rate proportional to the entire compensation for the study. If a subject becomes ill or physically injured because of participation in this study, the subject will be referred for treatment.

4.6.3 Screening

The following procedures and assessments will be performed at Screening (Day -28 to -2):

- Obtain informed consent/HIPAA authorization. The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding.

After informed consent is obtained:

- Review inclusion/exclusion criteria to confirm subject eligibility
- Record demographic information

- Measure height, weight, and calculate body mass index
- Perform serology screening (HIV antigen/antibody [Ag/Ab] Combo 1/2, HepC antibody, HBsAg)
- Record medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform urine cotinine test
- Perform a serum pregnancy test (female subjects only)
- Perform FSH measuring (postmenopausal [i.e., without menses for two years] female subjects only)
- Record prior medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a complete physical examination, including assessment of participant's Mallampati score
- Examine pupils for shape and reactivity
- Perform Duffin's rebreathing under hyperoxic and hypoxic conditions

4.6.4 Study Periods

This study will be a randomized, double blind, three period crossover study. This study will include three 21-day treatment periods with a 3-week washout between each period. Participants will be confined to the study clinic from day -1 until the first day of each washout period. There will be a three week washout between each period. Subjects will be discharged from the study after completion of all study procedures.

4.6.4.1 Check-In

The following procedures and assessments will be performed at Check-in [Day -1] and when participants check back into the clinic after each washout period:

- Perform/review results from SARS-CoV-2 rapid antigen test
- Review inclusion/exclusion criteria to confirm subject eligibility
- Review medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform urine cotinine test
- Perform a serum pregnancy test at all check-ins (female subjects only)
- Admit subject to the study clinic
- Randomization (after completion of check-in procedures or just before dosing on Day 1 of each study period)
- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature)
- Perform a safety 12-lead ECG (only at initial check-in of each study period)
- Perform a comprehensive physical examination
- Perform Duffin's rebreathing procedure under hyperoxic and hypoxic conditions
- Collect whole blood sample for CYP2D6 genotype/phenotype (only at check-in for first study period)

4.6.4.2 Treatment

The following procedures and assessments will be performed during the treatment period according to the Schedule of Events (Appendix A):

- Monitor for AEs
- Record concomitant medications
- Perform clinical laboratory tests at specified time points
- Administer study drug according to the randomization schedule following all other pre-dose examinations and specimen collection

- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature). If vital signs are scheduled at the same time as another event, vital signs will be measured after the ECG, but before blood sample collection
- Perform safety 12-lead ECGs at timepoints specified in Section 4.7.2.4. If scheduled for the same time, safety 12-lead ECGs will always be performed before vital sign measurement and blood sample collection
- Perform pulse oximetry monitoring as specified in Appendix A – Schedule of Events
- Perform rebreathing assessments following Duffin's rebreathing procedure at timepoints specified in Section 4.7.2.1. Preparatory steps for the Duffin Rebreathing procedure (i.e., relaxation, hyperventilation) should begin prior to the scheduled sample time. Timing of sample collection is planned so that the PD assessment begins at the specified time and a 3- to 15-minute window is planned for completion of the procedure.
- Perform pupillary assessments as specified in Appendix A – Schedule of Events
- Perform sedation assessments as described in Section 4.7.2.3 (Ramsey Sedation Scale and Visual Analogue Scale)
- Collect PK blood samples (6 mL) at timepoints specified in Section 4.7.1.1

4.6.4.3 Washout

After each period, there will be a three week washout period. Prior to discharge for each washout period, the following procedures will be performed:

- Record concomitant medications
- Monitor for AEs
- Perform targeted physical exam
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature)
- Collect PK blood samples (6 mL) at timepoints specified in Section 4.7.1.1
- Pre-discharge sedation assessment

When subjects check-in after each washout period, the procedures and assessments specified in Section 4.6.4.1 will be performed.

4.6.4.4 Discharge (or Early Termination)

The following procedures and assessments will be performed before a participant is discharged at the end of the study or at early termination:

- Record concomitant medications
- Monitor for AEs
- Measure height, weight, and calculate body mass index
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry and oral body temperature)
- Collect PK blood samples (6 mL) at the timepoints specified in Section 4.7.1.1 (only day 21 of each study period)
- Assess for pre-discharge sedation
- Perform a safety 12-lead ECG
- Perform a complete physical examination
- Discharge subject from the study clinic after completion of all study procedures

4.7 Study Procedures

4.7.1 Pharmacokinetic Assessments

4.7.1.1 Pharmacokinetic Sample Collection

Pharmacokinetic blood samples will be collected on the following days and at the approximate times for each study period:

- Day 5, 6, 11, 12, 20, and 21: 0 (pre-dose), 3, 4, 5, 8 hr (and 24 hrs for Day 6, 12, and 21)
- Day 2 and 16: 0 (pre-dose), 2, 3, 4, 5, 6, 8, 12, 24 hr

Blood samples will be collected by direct venipuncture or by inserting an IV catheter. There will be a total of 51 PK samples per participant for each study period (Total of 153 PK samples per participant for the entire study duration).

Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

Collection of the PK samples on the ECG collection days (Days 2 and 16 of each period) will be time-matched to the ECG extractions; therefore, the subjects will be supine for approximately 10 minutes before the defined time points and for approximately 5 minutes after for ECG extraction/PK sample collection. The PK sample will be collected after the

ECG extraction to avoid changes in autonomic tone associated with the psychological aspects of blood collection and the reduction in blood volume subsequent to blood collection. A 5-minute window for PK sample collection is allowed after the ECG extraction window.

4.7.1.2 Pharmacokinetic Specimen Handling

The PK blood samples (6 mL each) will be collected into tubes containing K_2EDTA, inverted several times to mix the blood with the anticoagulant, and placed in an ice bath. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes, at 3000 RCF, at 4°C, by a study team member.

The plasma will be separated using a disposable plastic pipette and equally aliquoted into duplicate cryotube vials labeled as Aliquot A (primary) and Aliquot B (backup). The plasma samples will be appropriately labeled and stored frozen at -70°C or below within 30 minutes after aliquoting until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

The Aliquot A samples (primary) will be shipped first, on dry ice, to the bioanalytical laboratory at FDA for processing when requested by the sponsor. The Aliquot B samples (backup) will be held for a second shipment after completion of all Aliquot A sample shipment(s) and the timing of the Aliquot B shipment will be communicated by the sponsor. None of the PK blood samples will be stored at the clinical facility for future use.

4.7.1.3 Pharmacokinetic Parameters

The following exploratory PK parameters will be determined for oxycodone and oxymorphone (days 6, 12, and 21) and paroxetine, escitalopram, and escitalopram metabolites (days 2, 5, 6, 11, 12, 16, 20, and 21):

- Maximum observed plasma concentration (C_{max})
- Area under the plasma concentration time curve (AUC)
- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})
- Terminal half-life ($t_{1/2}$)
- Accumulation ratio (for escitalopram and paroxetine)

4.7.2 Pharmacodynamic Assessments

4.7.2.1 Duffin Rebreathing Assessments

Training on the Duffin's rebreathing procedure will be performed to exclude any subjects who cannot tolerate the procedure.

During Duffin's rebreathing procedure, subjects will be placed in an upright position (90^{o} angle) on a bed and connected to the respiration measurement set up. Subjects will wear a facemask and breathe from a pre-filled rebreathing bag containing approximately 5 L of either a hyperoxic-hypercapnic (24% O_2, 6% CO_2, N_2 balanced) or hypoxic-hypercapnic (6% O_2, 6% CO_2, N_2 balanced) gas mixture. During this study, subjects will be asked to breath room air for 5 minutes (relaxation stage) and then to hyperventilate (primarily through deep breathing) room air voluntarily for 5 minutes (hyperventilation stage). Thereafter, subjects will be switched to the rebreathing bag and perform rebreathing assessments at one of the 2 different isoxic end tidal PO_2, i.e., 150 mmHg or 50 mmHg. The isoxia at 150 mmHg or 50 mmHg end-tidal PO_2 will be maintained by providing a computer controlled flow of 100% O_2 to the rebreathing bag.

Subjects will perform one hyperoxic and one hypoxic rebreathing procedure at check-in of each study period and a total of 4 rebreathing procedures (three hyperoxic and one hypoxic) each day on Days 5, 6, 11, 12, 20, and 21 of each study period. Rebreathing procedures will be performed while fasting and will occur at 0, 4, and 5 hours post-doses on assessment days (hyperoxic at 0, 4 and 5 hours; a hypoxic assessment will be performed after the 5 hour hyperoxic assessment). Participants can communicate to staff to discontinue an assessment due to discomfort at any time. Additional considerations for discontinuing a rebreathing procedure are described in a separate SOP for the Rebreathing Procedure.

Each rebreathing procedure (relaxation, hyperventilation, and rebreathing) should take approximately 13-15 minutes. Data collected throughout the procedure includes ventilatory flow and volumes as well as percent gas compositions of O_2 and CO_2, which are used to calculate multiple physiological variables.

Data from the rebreathing procedure will be acquired using the Hans Rudolph SmartLab^{TM} Data Acquisition System. Collected data will be analyzed using separate statistical software. Interim data analyses may be performed after each cohort of participants have completed. If reproducibility is acceptable and no procedural changes are needed, additional lead-in cohorts may not be enrolled. If any study procedure element changes are needed, they may be made and additional participants may be enrolled.

The clock on the rebreathing recorder will be confirmed to be consistent with the study clock at the clinical site; this will be confirmed on each study day. Preparatory steps for the Duffin's rebreathing procedure (i.e., relaxation, hyperventilation) should begin prior

to the scheduled sample time. Timing of sample collection is planned so that the rebreathing assessment begins at the specified time. Additional details on the Duffin's rebreathing procedure is described in a separate SOP.

The following physiologic measurements will be assessed multiple times throughout the study:

- Minute ventilation
- Tidal volume
- Respiratory rate
- End-tidal PCO_2
- End-tidal O_2
- Oxygen saturation (SpO_2)
- Heart rate

4.7.2.2 Pupillary Assessments

At Screening, subjects will be evaluated to ensure they meet requirements necessary for pupillary assessments (e.g., pupils are reactive and not misshapen). During the study, a pupillometer (a hand-held optical scanner) will be used to measure pupil size and dynamics in response to a light stimulus. Pupillary assessments will be performed before and after each rebreathing period (except not in between the hyperoxic and hypoxic paired assessment) while the ambient light is kept to a consistent level. The rubber cup of the pupillometer will be placed on the right eye. The actual data recording only takes seconds.

4.7.2.3 Sedation Assessments

The Ramsey sedation scale will be completed by an observer prior to the start of rebreathing assessment.

- Level 1: Patient awake, anxious and agitated or restless, or both
- Level 2: Awake, cooperative, orientated, and tranquil
- Level 3: Patient awake and responds to commands only
- Level 4: Asleep, brisk response to light glabellar tap or loud auditory stimulus
- Level 5: Asleep, sluggish response to light glabellar tap or loud auditory stimulus
- Level 6: Asleep, no response to light glabellar tap or loud auditory stimulus

At the same time as performing the Ramsey sedation scale, a visual analog scale will be administered to subjects by having subjects mark on a 100 mm perpendicular line how sedated they are from 'awake and alert' to 'very sedated' (i.e. 0 and 100 on the scale,

respectively). The distance from the left end of the line to the perpendicular mark will be measured in mm.

4.7.2.4 ECG Assessments

ECG assessments will be performed on the following study days and time of each study period:

- Day 1: 0 (pre-dose) hr
- Day 2 and 16: 0 (pre-dose), 2, 3, 4, 5, 6, 8, 12, 24 hr

Ten second 12-lead ECG tracings with the most stable heart rate and highest signal-to-noise ratio will be extracted in at least 3 replicates at ECG extraction time points during each treatment period. During all ECG extraction periods subjects will be placed in a supine position. Three ECG extractions will be performed for a baseline measurement before the first oral dose on day 1 of each treatment period. The other ECG extractions/period time matched to the PK samples will be obtained before the PK sample collection time. Subjects will be isolated (not able to see each other) in a quiet environment that is free from external stimuli.

4.7.3 Safety Assessments

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings.

4.7.3.1 Adverse Events

4.7.3.1.1 Adverse Event Definitions

An AE is defined as any untoward and/or unintended sign, including an abnormal clinical laboratory finding, symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug. Events or conditions that increase in frequency or severity during or as a consequence of use of a drug in human clinical trials will also be considered AEs.

A treatment emergent adverse event (TEAE) is defined as an AE that begins after study drug administration.

An unexpected AE is any AE having a specificity or severity not consistent with the current investigator’s brochure for the study drug(s).

A serious adverse event (SAE) is defined as any AE occurring at any dose that meets the following criteria:

- Results in death,
- Is life threatening,
- Requires hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability or incapacity,
- Results in a congenital anomaly/birth defect due to exposure prior to conception or during pregnancy, or
- Is an important medical event that may not meet the previous criteria but, based upon appropriate medical judgment, jeopardizes the subject or requires medical or surgical intervention to prevent one of the outcomes listed previously.

4.7.3.1.2 Adverse Event Reporting

The recording of AEs will begin after the subject signs the informed consent form and will continue until discharge (or early termination). All AEs, whether serious or nonserious and whether or not related to the study drug, must be recorded in the eCRF. Study subjects will be instructed to warn study staff if he or she has any unexpected symptoms. In addition, all subjects will receive a reminder telephone call approximately 24 h before Check-in.

Any SAE (whether expected or unexpected) must be entered into the eCRF system and reported by email to the medical monitor or designee using the SAE Reporting Form within 24 h of the investigator or study clinic staff becoming aware of the event. It is the responsibility of the investigator to report all SAEs to the medical monitor, to provide the most complete report possible, and to assess each SAE for its relationship to the study drug. The investigator is responsible for obtaining follow-up information on all SAEs and submitting follow-up SAE data. Any unexpected SAEs must be reported promptly to the investigator's IRB as per the IRB's requirements.

In the event of a fatal or life-threatening SAE, the sponsor will notify the appropriate FDA authorities within 7 calendar days of receipt of the report. The sponsor will follow all 7-day alert reports with a written report within 10 working days of receipt of the case. Serious AE cases that concern nonfatal, nonlife-threatening events that are unexpected and at least possibly related to the study drug will be submitted in writing to the FDA within 10 working days of receipt.

Furthermore, any AEs that are not expected, occur at a higher frequency, or would require modification of the study protocol and/or informed consent must be reported to the FDA within 10 working days.

Adverse events that are assessed by the investigator as possibly or probably related to the study drug will be followed until they resolve or stabilize. All SAEs will be followed until resolution.

4.7.3.1.3 Assessment of Severity

The investigator will assess the severity of each AE using the following scale:

- Mild: The subject is aware of the AE but is still able to perform all activities; minimal or no medical intervention or therapy is required.
- Moderate: The subject has to discontinue some activities due to the AE; minimal or no medical intervention or therapy is required.
- Severe: The subject is incapacitated by the AE and is unable to perform normal activities; significant medical intervention or therapy is required, and hospitalization is possible.

4.7.3.1.4 Assessment of Causality

The investigator will assess the causal relationship/relatedness of each AE to the study drug using the following scale:

- Not Related: Onset of the AE has no reasonable temporal relationship to administration of the study drug, a causal relationship to administration of the study drug is biologically implausible, or the event is attributed to an alternative etiology.
- Unlikely Related: Onset of the AE has a reasonable temporal relationship to study drug administration and although a causal relationship is unlikely, it is biologically plausible.
- Possibly Related: Onset of the AE has a strong temporal relationship to administration of the study drug, cannot be explained by the subject's clinical state or other factors, and a causal relationship is biologically plausible.
- Probably Related: Onset of the AE shows a distinct temporal relationship to administration of the study drug that cannot be explained by the subject's clinical state or other factors, the AE is a known reaction to the product or chemical group, or can be predicted by the product's pharmacology.

4.7.3.1.5 Pregnancy

A serum pregnancy test will be performed for female subjects at the time points presented in the Schedule of Events (Appendix A). If a subject becomes pregnant while on the study, this should be reported immediately to the investigator, the subject will be withdrawn from the study and the medical monitor and the subject will be instructed to

follow-up with his or her personal physician. All pregnancies are to be reported as an AE and followed for outcome.

4.7.3.2 Clinical Laboratory Tests

Clinical laboratory and diagnostic screening tests will be performed at the time points presented in the Schedule of Event and will be collected in accordance with acceptable laboratory procedures. Clinical laboratory testing will be performed by the clinical study contractor(s). The clinical laboratory tests that will be performed are presented in Table 4-3. Unused clinical laboratory test samples will not be stored for future use.

Table 4-3: Clinical Laboratory Tests & Diagnostic Screening Tests

Hematology	**Serum Chemistry**	**Urinalysis**
Hematocrit Hemoglobin Platelet count Red blood cell count White blood cell count (with automated differential)	Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bicarbonate Bilirubin (total, direct, and indirect) Blood urea nitrogen Calcium Chloride Creatinine (including calculated creatinine clearance) Glucose Lactate dehydrogenase Magnesium Phosphorus Potassium Sodium Total protein Uric acid	Appearance Bilirubin Blood Color Glucose Ketones Leukocyte esterase Microscopic examination: red blood cells, white blood cells, epithelial cells, bacteria, crystals, and casts (if present) Nitrite pH Protein Specific gravity Urobilinogen
Diagnostic Screening Tests:		
Serum	**Urine**	**Whole Blood**
Serology (human immunodeficiency virus Ag/Ab Combo 1/2, hepatitis C virus antibody, and hepatitis B surface antigen) **Female Subjects Only** Human chorionic gonadotropin (for pregnancy) Follicle-stimulating hormone	Drug screen including: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, methadone, and cotinine	Cytochrome P450 2D6 genotype (only performed at check-in for the first study period)
Other		
SARS-CoV2 rapid antigen test		

Clinical laboratory results will be reviewed by the investigator or designee together with data in the eCRF. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant, the subject will be instructed to follow-up with his or her personal physician. The investigator or designee may repeat the clinical laboratory tests if deemed appropriate. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

4.7.3.3 Vital Sign Measurements

Vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature) will be measured using an automated device at the time points presented in the Schedule of Events (Appendix A). The subject should be in a supine position, if possible, for a minimum of 5 minutes before vital signs are measured.

4.7.3.1 Pulse Oximetry

Continuous pulse oximetry monitoring will be performed on rebreathing days (Days 5, 6, 11, 12, 20, and 21 of each study period) specified in the Schedule of Events (Appendix A).

4.7.3.2 Safety 12-lead Electrocardiograms

Safety 12-lead ECGs will be obtained with the subjects in the supine position for a minimum of 10 minutes before recording. ECGs will be overread by the Principal Investigator or designee (e.g., a medically qualified subinvestigator). If an abnormality is observed, the subject will be instructed to follow-up with his or her personal physician.

4.7.3.3 Physical Examinations

A complete physical examination will be performed at the time points presented in the Schedule of Events (Appendix A).

The complete physical examination will include, but not be limited to, assessments of the head, eyes, ears, nose, throat, skin, thyroid, nervous system, respiratory system, cardiovascular system, abdomen (liver and spleen), lymph nodes, and extremities. Height, weight (without shoes and wearing the lightest possible clothing), and calculation of body mass index will be performed at Screening and check-out.

If a clinically significant abnormality is observed upon physical examination, the subject will be instructed to follow-up with his or her personal physician.

4.7.4 Demographics and Medical History

Demographic data (date of birth, gender, race, and ethnicity) will be collected at Screening.

Each subject will provide a complete medical history at Screening that will be reviewed at Check-in. Specific information relating to any prior or existing medical conditions/surgical procedures will be recorded in the subject's eCRF.

4.8 Study Treatments

4.8.1 Dose Strategy

Prior data with oxycodone alone using 20 mg doses, showed significant decreases in VE55 from baseline.[5-6] Our previous study that administered 10 mg oxycodone showed approximately 5 L/min decrease with oxycodone alone compared to baseline.[20] The goal with the oxycodone dose is to have an effect on the hypercapnic ventilatory response with oxycodone alone, but not too high (for safety considerations) as it will be combined with a SSRIs that may also have effects on ventilation.

Paroxetine dosing in labeling starts at 20 mg/day and allows titration up to a maximum of 60 mg/day for obsessive compulsive disorder (panic disorder starts at 10 mg/day and allows titration up to 60 mg/day). Dosing to 60 mg/day will allow assessment of effects on ventilation alone or combined with oxycodone under clinically relevant exposures

Escitalopram dosing in labeling starts at 10 mg/day and allows titration up to 20 mg/day for treatment of major depressive disorder and treatment of generalized anxiety disorder. The study will administer up to 30 mg/day, which is above the maximal labeled dose of escitalopram (20 mg/day), but doses up to 30 mg/day have been evaluated in a thorough QT study. Escitalopram exposure with 30 mg/day is similar to the steady state concentration expected in CYP2C19 poor metabolizers following a therapeutic dose of 20 mg/day.

4.8.2 Treatments Administered

For all drugs, standard drug doses or doses used in prior studies in healthy participants will be used in this study. Multiple doses of the assigned study drug will be administered to each subject during the treatment period. Oxycodone, paroxetine, escitalopram, ondansetron, and placebo will be administered orally. Subjects will complete each of the three treatments (A, B, and C):

- Treatment A: Placebo on Days 1-5; Placebo + Oxycodone (10 mg IR) on Day 6; Placebo on Days 7-11; Placebo + Oxycodone (10 mg IR) on Day 12; Placebo on Days 13-20; Placebo + Oxycodone (10 mg IR) on Day 21
- Treatment B: Paroxetine (40 mg) on Days 1-5; Paroxetine (40 mg) + Oxycodone (10 mg IR) on Day 6; Paroxetine (60 mg) on Days 7-11; Paroxetine (60 mg) + Oxycodone (10 mg IR) on Day 12; Paroxetine (60 mg) on Days 13-20; Paroxetine (60 mg) + Oxycodone (10 mg IR) on Day 21

- Treatment C: Escitalopram (20 mg) on Days 1-5; Escitalopram (20 mg) + Oxycodone (10 mg IR) on Day 6; Escitalopram (30 mg) on Days 7-11; Escitalopram (30 mg) + Oxycodone (10 mg IR) on Day 12; Escitalopram (30 mg) on Days 13-20; Escitalopram (30 mg) + Oxycodone (10 mg IR) on Day 21

Paroxetine, escitalopram, or placebo will be administered at time 0 on each day. Oxycodone dosing will occur at 3 h on days 6, 12, and 21. Participants will also receive ondansetron 4 mg 30 min before oxycodone administration on days 6, 12, and 21.

Study drugs will be administered by a clinical research nurse on the study clinic floor at the subject's bedside. The pharmacist and investigator will be available if needed during study drug administration. Oral study drugs will be administered with 240 mL of room-temperature water.

4.8.3 Dose Selection and Schedule

Oxycodone: The oxycodone dose is 10 mg IR. Oxycodone dose selection is based on previous published results where oxycodone was administered alone or in combination using rebreathing procedures in healthy volunteers. Two studies by van der Schrier and others evaluated oxycodone 20 mg alone or in combination with ethanol.[5-6] Sedation levels in the ethanol study in particular, which included a higher dose of oxycodone and was performed in the young and elderly, were moderate and well addressed by mild stimulation of the subjects. Varying dose combinations of oxycodone and morphine (total combined doses of 15 mg) have been safely evaluated using a similar system.[7] Our previous study with oxycodone 10 mg IR showed that small (approximately 5 L/min) but detectable effects on hypercapnic ventilation could be safely observed with this dose with minimal effects on baseline ventilation.[20]

Paroxetine: The paroxetine dose is 40 mg IR for Days 1-6 and 60 mg IR for Days 7-21. Paroxetine dose selection was based on available safety data from the original development program and to achieve sustained steady state exposures of paroxetine at the highest approved paroxetine dose for at least one week before the day 20 and 21 assessments. Target dosing range for paroxetine is 20-60 mg/day depending on the indication. Initial starting doses in labeling for general anxiety disorder, social anxiety disorder, posttraumatic stress disorder, obsessive compulsive disorder, and major depressive disorder are 20 mg/day with dose increases of 10-mg/day made in intervals of 1 week. Paroxetine has been administered at 30 mg/day for 30-days in healthy volunteers and up to 60-mg as a single dose in healthy volunteers.[51]

Escitalopram: The escitalopram dose is 20 mg for Days 1-6 and 30 mg for Days 7-21. Escitalopram dosing in labeling starts at 10 mg/day and allows titration up to 20 mg/day for treatment of major depressive disorder and treatment of generalized anxiety disorder. The study will administer up to 30 mg/day, which is above the maximal labeled dose of

escitalopram (20 mg/day), but doses up to 30 mg/day have been evaluated in a thorough QT study for a comparable duration of time. Escitalopram exposure with 30 mg/day is similar to the steady state concentration expected in CYP2C19 poor metabolizers following a therapeutic dose of 20 mg/day.[55]

Ondansetron: The ondansetron dose is 4 mg orally given 30 minutes before oxycodone administration. Up to two additional doses of IV ondansetron 4 mg can be given in a day for nausea/vomiting. The selected ondansetron dose is less than the recommended dosing for labeled indications of nausea and vomiting associated with highly or moderately emetogenic cancer chemotherapy, radiotherapy, or postoperative nausea or vomiting.

Naloxone: The naloxone dose is 0.4 mg administered intravenously, as needed, for respiratory depression as deemed necessary by the investigator. Naloxone may be administered as a rescue medication for opioid-induced respiratory depression.

4.8.4 Method of Assigning Subjects to Treatment Sequence

4.8.4.1 Randomization Process

The project biostatistician will create the specifications that will be used to generate the randomization schedule. The specifications will be based on the protocol requirements and appropriate statistical programming with consideration for study design, number of treatments, number of subjects planned for enrollment, stratification, and blocking.

Based on these specifications, the project biostatistician (or designee) will generate a dummy randomization schedule. The schedule is generated in R.

The project biostatistician (or designee) distributes the ‘dummy’ randomization schedule to specified personnel for review. Any change (e.g., change in block size, change in stratification levels) that requires an update to the specifications will reset this process. Minor changes (e.g., display formatting) will not require a change to the specifications.

After the approval of the ‘dummy’ randomization schedule, the project biostatistician (or designee) transfers the program used to generate the ‘dummy’ schedule to the randomization biostatistician (unblinded), who is an independent party and will not be participating in any programming or statistical decisions for the study before breaking the blind. No transfer is necessary if the unblinded randomization biostatistician also created the ‘dummy’ randomization.

The randomization biostatistician is responsible for generating the final randomization schedule. The output is sent only to designated unblinded recipients, who will maintain a secured digital and printed copy for their use.

Archival of the programs and output is accomplished by the creation of an encrypted, password-protected ZIP file containing the program and output file(s). The ZIP file is copied to a secure storage drive on the sponsor's site.

Randomization will occur after informed consent is obtained, either after completion of check-in procedures on Day -1 or just before dosing on Day 1. Approximately 25 healthy male and female subjects are planned for enrollment. Thus, a maximum of 25 subjects will be exposed to study drugs and procedures during the study.

Enrolled subjects will be randomly assigned to 1 of 6 different treatment sequences consisting of the treatment groups are presented in Table 4-2.

All randomization information will be secured and housed in a locked storage area, accessible only by the randomization personnel and the assigned pharmacist and his or her verifier.

4.8.5 Identity of Study Drugs

Oxycodone HCl (referred to throughout this document as oxycodone) is indicated for the management of pain severe enough to require an opioid analgesic and for which alternative treatments are inadequate. Oxycodone tablets are available as 5, 10, 15, 20, or 30 mg strengths. The tablets are designed for immediate release of the drug where about 60% to 87% of the drug reaches systemic circulation in comparison to a parenteral dose. Oxycodone HCl has a molecular weight of 351.82 and the molecular formula $C_{18}H_{21}NO_4 \bullet HCl$. The physical form is a white odorless crystalline powder derived from the opium alkaloid, thebaine. Oxycodone dissolves in water (1 g in 6 to 7 mL) and is considered slightly soluble in alcohol.[53]

Paroxetine HCl (referred to throughout this document as paroxetine) is indicated for the treatment of major depressive disorder. Paroxetine HCl has a molecular weight of 374.8 and the empirical formula is $C_{19}H_{20}FNO_3 \bullet HCl \bullet 1/2H_2O$. Paroxetine HCl is an odorless, off-white powder with a solubility of 5.4 mg/mL in water. Tablets are available for oral administration in 10, 20, 30, and 40 mg strengths.[48]

Escitalopram oxalate (referred to throughout this document as escitalopram) is indicated for acute and maintenance treatment of major depressive disorder and acute treatment of generalized anxiety disorder. Escitalopram is the pure S-enantiomer of the racemic bicyclic phthalane derivative citalopram. Escitalopram oxalate has a molecular weight of 414.4 and the empirical formula is $C_{20}H_{21}FN_2O \bullet C_2H_2O_4$. Escitalopram oxalate is a fine, white to slightly-yellow powder and is freely soluble in methanol and dimethyl sulfoxide, soluble in isotonic saline solution, sparingly soluble in water and ethanol, slightly soluble in ethyl acetate, and insoluble in heptane. Tablets are available for oral administration in 5, 10, and 20 mg strengths.[49]

Ondansetron is indicated for the prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy, including cisplatin greater than or equal to 50 mg/m2, initial and repeat courses of moderately emetogenic cancer chemotherapy, radiotherapy in patients receiving either total body irradiation, single high-dose fraction to the abdomen, or daily fractions to the abdomen, and prevention of postoperative nausea and/or vomiting. The empirical formula is C18H19N3O●HCl●2H2O, representing a molecular weight of 365.9. Ondansetron hydrochloride dihydrate is a white to off-white powder that is soluble in water and normal saline. Ondansetron is available for oral administration as 4 and 8 mg tablets. Ondansetron hydrochloride is supplied as 20 mL multi-dose vials, 2 mg/mL.[56]

Naloxone hydrochloride injection is indicated for the complete or partial reversal of opioid depression, including respiratory depression, induced by natural and synthetic opioids. Naloxone hydrochloride is also indicated for the diagnosis of suspected or known acute opioid overdosage. Naloxone hydrochloride, an opioid antagonist, is a synthetic congener of oxymorphone, with a molecular weight of 363.84 and molecular formula of C19H21NO4●HCl. Naloxone hydrochloride injection may be diluted for intravenous infusion in 0.9% sodium chloride injection or 5% dextrose injection. The addition of 2 mg of naloxone hydrochloride in 500 mL of either solution provides a concentration of 0.004 mg/mL. Mixtures should be used within 24 h. After 24 h, the remaining unused solution must be discarded. Naloxone hydrochloride injection is supplied as 10 mL multi-dose vials, 4 mg/10 mL (0.4 mg/mL).[52]

Placebo capsules containing only cornstarch will be used as the placebo oral control in this study.

4.8.6 Management of Clinical Supplies

4.8.6.1 Study Drug Packaging and Storage

The active study drugs will be obtained from commercial sources. Storage instructions for the active study drugs are as follows:

- Oxycodone tablets should be stored at 25°C (77°F) with excursions permitted from 15° to 30°C (59° to 86°F) and should be protected from moisture.[53]
- Paroxetine tablets should be stored between 15° and 30°C (59° and 86°F).[54]
- Escitalopram tablets should be stored between 20° and 25°C (68° and 77°F); excursions permitted to 15° and 30°C (59° and 86°F).[55]
- Naloxone hydrochloride injection should be stored at 20° to 25°C (68° to 77°F).[58]
- Ondansetron tablets should be stored at 2° to 30°C (36° to 86°F).[56]

- Ondansetron injection should be stored at 20° to 25°C (68° to 77°F).[59]

Placebo capsules will be supplied by the clinical site, stored at controlled room temperature (15°C to 30°C; 59°F to 86°F), and protected from light and moisture.

4.8.6.2 Study Drug Accountability

Good clinical documentation practices will be employed to record the receipt, storage conditions, accountability, and use or return of the study drug. The study drug will be stored in a secure location with access to the study personnel who will be managing the storage, dispensing, and accountability of the study drug.

Upon completion or termination of the study, final accountability review by the study monitor, and written authorization from the sponsor, all unused and/or partially used study drug should be returned or destroyed at the study clinic. It is the investigator's responsibility to ensure that the sponsor has provided written authorization for study drug disposal, the disposal process follows the study clinic's standard operating procedures, and appropriate records of the disposal are documented and maintained. No unused study drug may be disposed until fully accounted for by the study monitor (or designee). Documentation of unused study drug should include subject number, medication identity (medication #, period #), date, and quantity of study drug used.

4.8.7 Blinding

The study will be double-blind, and the blind will be maintained through a randomization schedule held by the dispensing pharmacist. Treatments (escitalopram, paroxetine, or placebo) will be over-encapsulated. The pharmacist (and designated staff member responsible for confirmation of study drug dose) will be unblinded to subject treatment assignment; however, the pharmacist will not perform any study procedures other than study drug preparation and dispensing.

Additional details regarding blinding can be found in the Spaulding Blinding SOP which will be followed to ensure the blind is maintained throughout the study.

4.8.7.1 Breaking the Blind

The study drug blind will not be broken by the investigator or designee unless information concerning the study drug is necessary for the medical treatment of the subject. For unblinding a subject, the randomization information for unblinding can be obtained by contacting the dispensing pharmacist. The sponsor or medical monitor must be notified immediately if the study drug blind is broken. The date, time, and reason that the blind was broken will be recorded in the source documents. If the blind is broken by the investigator or designee, the study drug must be stopped immediately, and the subject

must be withdrawn from the study. Data or specimens already collected from subjects who discontinue prematurely and for whom the blind is broken will be made available for analysis if needed.

4.8.8 Treatment Compliance

At Screening, as part of the inclusion criteria, it will be confirmed that subjects are able to comply with the protocol-defined procedure of injecting subcutaneous study drug. All doses of the study drug will be administered in the study clinic either under direct observation of or administered by clinic personnel and recorded in the eCRF. If a subject vomits after dosing, the event will be documented as an AE. The decision to replace any subject who vomits after dosing will be made as described in Section 4.5.1.

4.8.9 Prior and Concomitant Medications

Subjects are prohibited from using any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer), or complementary and alternative medicines within 28 days before the first dose of study drug. Subjects are prohibited from using any opioid or psychotropic drugs within 60 days of the start of the study. Subjects will be asked if they have used any of these substances and their responses will be recorded on the eCRF.

Subjects are also prohibited from currently participating in another clinical study of an investigational drug and may not have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.

Subjects must be instructed not to take any medications, including over-the-counter products, without first consulting with the investigator.

4.8.10 Subject Restrictions

Subjects are not allowed to use nicotine containing products (e.g., cigarettes, cigars, chewing tobacco, snuff, electronic cigarettes) within 6 weeks before Screening. In addition, subjects are not allowed to ingest alcohol, xanthine containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 24 h of check-in of all study periods. Subject must refrain from ingesting these throughout the entire study. Subjects are not allowed to use aspirin or NSAIDs within 14 days before the first dose of study drug. Subjects will be asked if they have used any of these substances and their responses will be recorded on the eCRF.

Subjects must be able to tolerate a controlled, quiet study conduct environment, including avoidance of music, television, movies, games, and activities that may cause excitement, emotional tension, or arousal during prespecified times (e.g., before and during ECG

extraction windows; before and during rebreathing procedures) throughout the duration of the study.

Subjects must be willing to comply with study rules; attempting to void at specified times; remaining quiet, awake, undistracted, motionless, and supine during specified times; and avoiding vigorous exercise as directed throughout the duration of the study.

Standardized meals will be served at consistent times relative to dosing, and no food or fluids will be served containing caffeine. Subjects will fast during days with rebreathing assessments (i.e., Day 5, 6, 11, 12, 20, and 21 of each period) until after the last rebreathing assessment. On ECG collection days (Day 2 and 16 of each period) subjects will have fasted for at least 8 hours prior to drug administration. Subjects should only eat meals and snacks that are provided during periods of their stay in the study clinic, and should consume each meal that is served at a reasonable pace (within 25 minutes). Outside of meal times, the subjects will only be allowed to intake water, which will be available ad libitum.

Due to current precautions being taken for COVID-19, the following restrictions will be in place:

- Subjects should be encouraged to wear masks except when in a private room without anyone else present or for a limited time for a study procedure (e.g., study drug administration or eating) when instructed by staff.
- Subjects must practice social distancing, which will include having a maximum of 2 subjects per room for overnight stays and access to common areas will be per clinical research site standards. While subjects are in house, meals will be served per clinical research site standards. Subjects will spend most of their time in their rooms except for specified times for walking in the halls (with masks recommended).
- Subjects must practice regular handwashing with soap and water, scrubbing hands for at least 20 seconds or with approved hand sanitizer as supplied by study staff.

Designated isolation rooms will be set up to segregate any participant(s) that develop any symptoms of concern while housed in the unit and COVID-19 testing will be done when deemed necessary by the Investigator. If new information becomes available, there could be other precautions that lead to additional restrictions that will be documented in the COVID-19 Risk Management Plan and/or the study specific COVID-19 Procedure Plan.

4.9 Statistical Methods

4.9.1 Sample Size

Approximately 25 healthy participants are planned for enrollment. A previous study (20 participants) with paroxetine showed a decrease in 10.2 L/min in VE55 for paroxetine (40

mg) combined with oxycodone (10 mg) compared to oxycodone alone after 5 days of dosing with paroxetine. Similarly, there was a 9.3 L/min decrease in paroxetine compared to placebo after 4 days of dosing with paroxetine. The standard deviation in assessments was approximately 6.5 L/min. Assuming a similar effect size with paroxetine and escitalopram at day 20 and 21, the planned number of participants allows for a 40% drop out rate while maintaining at least 90% power for separate one-sided tests at a 0.025 significance level with the multiple primary endpoint. Study sample size did not consider secondary endpoints.

4.9.2 Analysis Populations

The rebreathing analysis population will include all subjects who completed at least one rebreathing assessment on any day or at any timepoint. Specific information on analysis populations for different pharmacodynamic measures will be described in the Statistical Analysis Plan (SAP).

A rebreathing assessment will be considered complete if the subject makes it through the entire procedure at a specific timepoint, if there are no identifiable issues with how the procedure was conducted, and if the VE55 regression converges. Potential issues with how the procedure was conducted can included, but are not limited to, a leak from the system (e.g., substantially decreasing O_2 during rebreathing or no evidence of an increase in CO_2 during rebreathing) or inaccurate readings from the pneumotach (e.g., baseline minute ventilation readings less than 5 L/min).

Subjects in this population will be used for the planned primary and secondary analyses related to evaluating drugs effects on the ventilatory response to hypercapnia. If a subject does not contribute data from all treatments due to early discontinuations or other reasons, only those comparisons where the subject has all required data will be performed.

The PK population will include all subjects who receive study drug and have at least one estimable PK parameter after dosing.

The safety population will include all subjects who receive at least one dose of any of the study drugs.

4.9.3 General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings, but not included in the calculation of summary statistics. Demographic and baseline characteristics will be summarized overall and by treatment for all subjects.

4.9.4 Subject Disposition

The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized.

4.9.5 Demographics and Baseline Characteristics

Descriptive statistics will be used to summarize demographic and baseline subject characteristics. For continuous variables, the mean, median, standard deviation (SD), minimum, and maximum values will be reported. For categorical (nominal) variables, the number and percentage of subjects (or observations) will be reported.

4.9.6 Rebreathing Analyses

4.9.6.1 Primary Analysis

The primary objective is to study ventilatory effects of SSRIs (paroxetine or escitalopram) combined with an opioid (oxycodone) compared to an opioid alone. The primary endpoint is VE55 under hyperoxic conditions on day 21.

4.9.6.2 Data Analysis

Minute ventilation and end-tidal pCO_2 ($P_{ET}CO_2$) data from the rebreathing stage of the Duffin rebreathing procedure will be analyzed at the specified primary timepoints using piecewise linear regressions. The regressions will be used to predict VE55. VE55 when the SSRIs (paroxetine or escitalopram) are combined with oxycodone will be compared to VE55 with oxycodone alone.

Multiple primary analyses are planned for each drug. The first primary analysis will be VE55 on day 21 at 5 h (hyperoxic) between oxycodone with paroxetine or escitalopram compared to oxycodone alone. The second primary analysis will be VE55 on day 20 at 5 h (hyperoxic) between paroxetine or escitalopram compared to placebo. Since Day 20 and 21 are considered multiple primary endpoints, comparisons will have an adjustment for multiplicity using a Bonferroni correction (each day will be tested at a 0.025 significance level). No adjustments will be made for separate testing of paroxetine or escitalopram since the treatments are considered separate interventions in the study.

A linear mixed effects model will be developed using all study data from hyperoxic assessments. Fixed effects will include treatment, period, sequence, and baseline VE55 (i.e., VE55 obtained at 0 h on Day -1 of each treatment period). Subject will be included as a random effect on the intercept. If a subject's baseline VE55 for a treatment period is not available, then the baseline value for that period will be calculated based on the median of all other baseline values for that subject from other study periods. If a subject does not

have any completed baseline VE55 data, the subject will be assigned a baseline VE55 equal to the median of the population.

The Kenward-Roger approximation will be used to estimate denominator degrees of freedom for tests of fixed effects. Normality assumption will be verified using the Shapiro-Wilke test for normality. Homogeneity of variances will be verified using Levene's test. If the data in its original or transformed form does not satisfy assumptions for normality and homogeneity, a Wilcoxon signed-rank test will be used for all comparisons rather than a linear mixed effect model. In the event that the data does not satisfy assumptions for normality and homogeneity then a Wilcoxon signed-rank test will be used for comparisons.

To demonstrate an effect when an SSRI (paroxetine or escitalopram) is combined with oxycodone compared to oxycodone alone, the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments must not overlap 0 L/min. Similarly, to demonstrate an effect of paroxetine (or escitalopram) compared to placebo the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments must not overlap 0 L/min. Each comparison will be performed separately (i.e., 'trt' as 'paroxetine' or 'escitalopram'):

- H0: VE55oxy+trt - VE55oxy $\geq$ 0 L/min
- HA: VE55oxy+trt - VE55oxy < 0 L/min

For secondary endpoints, effects of SSRIs (paroxetine or escitalopram) with oxycodone compared to oxycodone alone will be performed on day 6 and day 12.

As additional secondary endpoints, effects of SSRIs (paroxetine or escitalopram) alone compared to placebo will be determined on day 5 and 11 using the same model. Additional details on testing of ventilation secondary endpoints is discussed in the SAP.

4.9.7 Pharmacokinetic Analysis

The PK parameters C_{max}, AUC_{0-t}, T_{max}, and K_{el} will be summarized using descriptive statistics (number of subjects, geometric mean, coefficient of variation [CV], mean, SD, median, minimum, and maximum) on Day 2, 5, 6, 11, 12, 16, 20 and 21 of each period for paroxetine, escitalopram, and escitalopram metabolites (Days 6, 12, and 21 for oxycodone and oxymorphone). The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. Geometric mean and individual concentration-time profiles will be presented in graphs.

4.9.8 PK/PD Modeling

A nonlinear-mixed effect pharmacokinetic/pharmacodynamic model will be developed for VE55 and baseline-adjusted VE55 versus time for all treatments. The model will be developed using modeling software.

Additional details on PK/PD modeling will be discussed in a separate Modeling Analysis Plan.

4.9.9 Electrocardiogram Analyses

The QT interval will be corrected for heart rate using Fridericia's formula ($QTc=QT/RR^{1/3}$). The J-T_{peak} interval will be corrected for heart rate using the formula ($J\text{-}T_{peakC} = J\text{-}T_{peakC}/RR^{0.58}$).[60] Baseline will be the mean of the 3 predose ECG extractions of Day 1.

Exposure-response analyses will be performed for the change-from-baseline in QTc (ΔQTc) and change from baseline in other ECG measurements (specified in ECG Analysis Plan), where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline. The concentration of the drug will be used as a covariate. Exposure-response analysis will be done following most recent best practices in concentration-QTc modeling.[61-62]

To assess the appropriateness of a linear model, normal QQ-plots for the residuals and plots of weighted residuals versus concentration and versus fitted values will be produced. A model with a quadratic term in concentration will be fitted and the quadratic term will be tested on the 2-sided 5% alpha level. In case of a significant quadratic term, nonlinear models, such as a log-linear model and an E_{max} model, will be investigated and the primary model will be selected based on the Akaike Information Criterion and plausibility arguments.

Unless the prespecified test procedure for linearity indicates otherwise, the primary analysis will be based on a linear mixed effects model implemented in R software, with ΔQTc as the dependent variable, drug plasma concentration and baseline QTc as continuous covariates, treatment and time point as categorical factors, and subject specific random effects for the intercept and slope. All post dose data will be used. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From the model, the slope (i.e., the regression parameter for the concentration) and the treatment effect will be estimated together with 2-sided 90% CIs.

The predicted mean placebo-adjusted change from baseline QTc (ΔΔQTc) at the observed geometric mean C_{max} (i.e., the product with the slope estimate + treatment effect [$\Delta QTc_{active} - \Delta QTc_{placebo}$]) and the 2-sided 90% CI of the estimate will be calculated.

Additional details will be specified in the ECG Analysis Plan, including for analysis of other ECG measurements.

4.9.10 Additional Analyses

4.9.10.1 Exploratory Respiratory Analyses

Baseline minute ventilation, ventilatory recruitment threshold, slope of the PCO_2-ventilatory response curve, and the extrapolated ventilatory recruitment threshold will be calculated for each subject at each rebreathing assessment. Data collected during the Duffin Rebreathing procedure will be used to determine the slope of the minute ventilation / $P_{ET}CO_2$ regression line. Resting ventilation, tidal volume, end-tidal PCO_2, and oxygen saturation will be determined using data from the relaxation portion of the Duffin Rebreathing procedure. Apneic events lasting > 10 s will be determined using data collected during relaxation and preparation portion of the Duffin Rebreathing procedure. An event is defined as the absence of inspiratory flow (as measured by the pneumotachograph) for at least 10 s during this period. These parameters will be summarized using descriptive statistics, and time courses summaries will be generated for all treatment groups. Differences in hypercapnic ventilatory response under hyperoxic or hypoxic rebreathing at the 5-h timepoint will be performed using paired t-test. These analyses will be considered hypothesis-generating and exploratory.

4.9.10.2 Pupillometry Analyses

Maximum pupil diameter before constriction and dynamic pupillary measurements after a light stimulus will be measured before and after each rebreathing assessment (except not in between the hyperoxic and hypoxic paired assessment). These parameters will be summarized using descriptive statistics, and time courses summaries of both scores will be generated for all treatment groups. Pupillary changes will be compared to baseline measurements and between treatments to evaluate the effect of different interventions on pupillary changes. In addition, time course pupillary changes will be compared to time course ventilatory changes across treatments to evaluate concordance between these measures when subjects receive different drugs and drug combinations. These analyses will be considered hypothesis-generating and exploratory.

4.9.10.3 Sedation Scores Analyses

Ramsay Sedation Scale is an observer-based assessment of sedation that will be collected for each subject as during the relaxation period of each Duffin Rebreathing procedure. In addition, subjects will be asked to provide their own assessment of sedation using the Visual Analog Scale during the same relaxation period. These are two measures for assessing an individual's level of sedation and will provide a subjective assessment of how sedation the subject is experiencing during the study. These parameters will be summarized using descriptive statistics, and time courses summaries of both scores will be generated for all treatment groups. These analyses will be considered hypothesis-generating and exploratory.

4.9.11 Safety Analyses

4.9.11.1 Adverse Events

All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. The incidence of TEAEs, organized by system organ class and frequency, will be summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided.

4.9.11.2 Clinical Laboratory Tests

Clinical laboratory results will be reviewed by the investigator or designee together with data in the eCRF. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant, the subject will be instructed to follow-up with his or her personal physician. The investigator or designee may repeat the clinical laboratory tests if deemed appropriate. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

Clinical laboratory results (hematology, serum chemistry, and urinalysis) will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum). Clinical laboratory results will be classified as normal or abnormal, according to the reference ranges of the individual parameter. The number and percentage of subjects with abnormal laboratory results will be provided. No statistical testing will be performed on clinical laboratory data.

4.9.11.3 Vital Sign Measurements

Vital sign measurements and changes from Baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point.

4.9.11.4 Pulse Oximetry

Continuous pulse oximetry will be performed on rebreathing days. Events requiring intervention from the staff or discontinuation from the study will be recorded in appropriate logs.

4.9.11.5 Safety 12-lead Electrocardiograms

12-lead ECGs will be obtained with the subjects in the supine position for a minimum of 5 minutes before recording. ECGs will be overread by a physician. If an abnormality is observed, the subject will be instructed to follow-up with his or her personal physician.

4.9.11.6 Physical Examinations

Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs.

4.9.11.7 Other Safety Data

All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided informed consent will be summarized for each subject.

4.9.12 Interim Analyses

No interim analyses are planned.

4.9.13 Missing Data

Missing data will not be imputed. Data that are excluded from the descriptive or inferential analyses will be included in the subject data listings. This will include data from subjects not in the particular analysis population, measurements from unscheduled visits, or extra measurements that may arise from 2 or more analyses of the biofluid sample at the same time point. Details on the handling of missing data will be further described in the SAP.

4.10 Data Quality Assurance

Completed eCRFs are required for each subject randomly assigned to treatment. Electronic data entry will be accomplished through the ClinSpark® remote electronic data capture system, which allows for on-site data entry and data management. This system provides immediate, direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records.

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system. The completed dataset and their associated files are the sole property of the sponsor and should not be made available in any form to third parties, except for appropriate governmental health or regulatory authorities, without written permission of the sponsor.

4.11 Data Sharing

De-identified subject-level data may be released to other researchers (including through a data warehouse or as a part of a publication) to enable secondary research. Additional secondary research may also be performed by the sponsor.

5. ETHICAL CONSIDERATIONS

5.1 Ethical Conduct of the Study

This study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, 1964 and later revisions, as well as, United States Title 45 Code of Federal Regulations (CFR) Part 46 GCP, and International Council for Harmonisation (ICH) guidelines describing technical requirements for registration of pharmaceuticals for human use.

5.2 Institutional Review Board (IRB)

The FDA Project Lead or investigator will provide the designated IRB with all required documents, including the study protocol and informed consent form. The study will not be initiated until appropriate IRB approval is obtained from the designated IRB. The investigator will provide the FDA Project Lead with copies of the approval documents for the protocol, informed consent form, and all recruiting materials. The designated IRB will also receive copies of any original or amended information sheets or pamphlets given to the study subject in support of the informed consent process and any advertisements or other recruitment material. Such materials will not be employed in the study before approval by the designated IRB.

Subjects will be informed that they have the right to contact the IRB or Office for Human Research Protections if they have any questions, concerns, complaints, or believe they have been harmed by the participation in this research study as a result of investigator negligence. Subjects will be given the address and phone number of the IRB.

6. ADMINISTRATIVE PROCEDURES

6.1 Responsibilities of the Investigator

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes may be reported to the IRB but will not result in protocol amendments.

6.1.1 Form FDA 1572

The investigator will complete and sign the Form FDA 1572.

6.1.2 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with the ICH E6(R2) and all applicable guidelines and regulations.

6.1.3 Reporting Requirements

By participating in this study, the investigator agrees to submit reports of SAEs according to the timeline and method outlined in the protocol (Section 4.7.3.1.2). In addition, the investigator agrees to submit reports to the IRB as appropriate. The investigator also agrees to provide the sponsor with an adequate report shortly after completion of the investigator's participation in the study.

6.1.4 Source Documentation

By participating in this study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories.

6.1.5 Retention of Records

The investigator agrees to keep the records stipulated in this protocol and those documents that include (but are not limited to) the study-specific documents, identification log of all participating subjects, medical records, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent form), copies of all eCRFs, query responses, and detailed records of drug disposition, to enable evaluations or audits from regulatory authorities, the sponsor, or its designees.

Furthermore, ICH 4.9.5 requires the investigator to retain essential documents specified in Section 8 of ICH E6(R2) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the clinical study site agreement between the investigator and sponsor.

Refer to the clinical study site agreement for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

6.1.6 Financial Disclosure and Obligations

The investigator is required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 45 CFR 46. In addition, the investigator must provide to the sponsor a commitment to update this information promptly if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.

Neither the sponsor nor the study clinic is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process.

6.2 Confidentiality and Disclosure of Data

All subjects will sign a HIPAA-compliant authorization form containing the mandated core elements and requirements before participation in this clinical study. The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to the sponsor's electronic data capture system database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes such as gender, age or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires that the investigator allow review of the subject's original medical records (source data or documents) by the study monitor, representatives from any regulatory authority (e.g., FDA), the sponsor's designated auditors, and the appropriate IRB. These medical records will include, but will not be limited to, clinical laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process.

Copies of any subject source documents that are provided to the sponsor must have personally identifiable information removed (i.e., subject name, address, and other identifier fields not collected in the subject's eCRF).

Data will be maintained and backed up in the electronic data capture system. All access to the data is protected by username and password, and each staff member and all sponsor staff will have separate access that requires a separate username and password. Access is only given to site staff and requested sponsor staff who have completed the appropriate training.

6.3 Subject Consent

Written informed consent in compliance with 45 CFR 46 will be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that

involves risk to the subject. An informed consent template may be provided by the sponsor to the study clinic. If any institution-specific modifications to study-related procedures are proposed or made by the study clinic, the consent should be reviewed by the sponsor or its designee or both before IRB submission. Once reviewed, the consent will be submitted by the investigator to the IRB for review and approval before the start of the study. If the informed consent form is revised during the course of the study, all active participating subjects must sign the revised form.

Before enrollment, each prospective subject will be given a full explanation of the study and be allowed to read the approved informed consent form. The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding. Once the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing the informed consent form.

The investigator will provide a copy of the signed informed consent form to the subject. The original form will be maintained in the subject's medical records at the site.

6.4 Data Collection

Full details of procedures for data collection and handling will be documented in the data management plan, which is initiated with the final protocol receipt. The data management plan is a changing document that evolves over the course of the study and is finalized by database lock.

6.5 Publications

No information related to or generated by this study will be released to the public until it has been reviewed by the sponsor. The sponsor shall own intellectual rights for the data and analysis resulting from this study. Authorship on publications will be determined by standard journal requirements.

7. STUDY MANAGEMENT

7.1 Monitoring

The sponsor or its designee will monitor the study to ensure that it is being conducted according to the protocol, GCP standards, and applicable region-specific requirements, and to ensure that study initiation, conduct, and closure are adequate. The investigators and the study clinic staff will be expected to cooperate fully with the study monitors and personnel or agents of the sponsor and be available during monitoring visits to answer

questions sufficiently and to provide any missing information. The investigators and their institutions will permit direct access to source data/documents for study-related monitoring activities, audits, IRB reviews, and regulatory inspections.

During any on-site visits, the study monitor will:

- Check and assess the progress of the study
- Review all informed consent forms
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution
- Verify that the facility remains acceptable
- Conduct study drug accountability

These monitoring activities will be done in order to verify that the:

- Data are authentic, accurate, and complete.
- The safety and rights of the subjects are being protected.
- The study is being conducted in accordance with the currently approved protocol (including any amendments), GCP, and all applicable regulatory requirements.

In addition, the sponsor, designated auditors, and government inspectors must be allowed access to eCRFs, source documents, and other study files that may be required to evaluate the conduct of the study.

7.2 Management of Protocol Amendments and Deviations

7.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent immediate hazard to the subject, must be submitted to the sponsor or designee and reviewed and approved by the local IRB before implementation. Amendments to the protocol must be submitted in writing to the investigator's IRB for approval before subjects are enrolled into an amended protocol.

7.2.2 Protocol Violations and Deviations

Any significant protocol deviations that the investigator or study clinic staff believes are of major importance (e.g., incorrect randomizations, subject enrolled but not eligible)

should be reported to the sponsor and the investigator's IRB as soon as possible. Significant protocol deviations may include the following:

- Deviations from the inclusion/exclusion criteria that may affect subject safety
- Deviations (omission or delay) of safety monitoring procedures
- Deviations in the administration of the study drug
- Deviations in obtaining informed consent

All subjects who are enrolled and receive the study drug, regardless of whether they have a major protocol violation, must continue to be followed for safety for all follow-up study visits.

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8. APPENDICES

Appendix A – Schedule of Events

Opioid and Sedative Drugs - Study 2 - Three Period Crossover (n=25)

	Screening	Check-In and Baseline	Treatment Period 1													Washout Period 1 (Check-out 1/ Check-in 2)	Treatment Period 2	Washout Period 2 (Check-out 2/ Check-in 3)	Treatment Period 3	Check-out
Study Day	-28 to -2	-1	1	2	3-4	5	6	7-10	11	12	13-15	16	17-19	20	21	22-41	42	43-63	64-83	84	85-105	106
Week			1	1	1	1	1	2	2	2	2-3	3	3	3	3	4-5	6	7-9	10-11	12	13-15	
Visit	1	2															3			4		
Recruitment/Screening - healthy	X																	Repeated Events as decribed in Treatment Period 1			Repeated Events as decribed in Treatment Period 1	
Screening Stipend	X																					
Screening Failures	X																					
In House Residency		1	1	1	2	1	1	4	1	1	3	1	3	1	1		1			1		
Meals		1	1	1	2	1	1	4	1	1	3	1	3	1	1		1			1		
Informed Consent	X																					
Medical History	X	X															X			X		
Height/Weight	X																					
Comprehensive Physical Exam	X	X															X			X		X
Targeted Physical exam																X			X			
Vital signs (including pulse oximetry)	1	1	1	1		1	1		1	1		1		1	1	1	1		1	1		1
Pre-discharge Sedation Assessment																X			X			X
ECG - single[1]	X	X																				X
ECG - triplicate			1	3								3										
Chemistry/hematology[1]	X	X															X			X		X
Basic chemistry (bicarb)						1			1					1								
Urinalysis	X	X															X			X		X
Urine drug screen + alcohol screen	X	X															X			X		
Urine cotinine test	X	X															X			X		
Pregnancy test[2]	X	X															X			X		
FSH (females only)	X																					
HIV test	X																					
Hepatitis test	X																					
COVID-19 Rapid Antigen Test		X															X			X		
Study Agent preparation & administration - oral			2	2	4	2	2	8	2	2	6	2	6	2	2							
Sedation Assessment (See protocol for time points)						3	3		3	3				3	3							
Duffin Rebreathing Procedure (See protocol for time points)	2	2				4	4		4	4				4	4		2			2		
Continuous Pulse Oximetry						1	1		1	1				1	1							
Plasma PK draw (See protocol for time points)				3		5	6		5	6		3		5	6							
CYP2D6 testing		X																				
Pupillometry Assessment						6	6		6	6				6	6							
Con Med/AE Assessment	1	1	2	1	1	1	1	4	1	1	3	1	3	1	1	1	1		1	1		1
eSource Management		X																				

1 Clinically notable results are repeated
2 Serum test

Appendix B – List of Abbreviations

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
Ag/Ab	antigen/antibody
AUC	area under the concentration-time curve
CDC	Center for Disease Control
CFR	Code of Federal Regulations
CI	confidence interval
C_{max}	maximum observed concentration
COVID-19	coronavirus disease of 2019
CO_2	carbon dioxide
CV	coefficient of variation
CYP	cytochrome P450
ECG	electrocardiogram
eCRF	electronic case report form
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HepC	hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
ICH	International Council for Harmonisation
IR	immediate release
IRB	Institutional Review Board
IV	intravenous
J-T_{peak}	early repolarization interval
J-T_{peakC}	heart rate-corrected J-T_{peak} interval
ΔJ-T_{peakC}	change-from-baseline in J-T_{peakC}
$\Delta\Delta$J-T_{peakC}	placebo-adjusted change-from-baseline J-T_{peakC}
K_{el}	elimination rate
Kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
Mg	milligram
NONMEM	nonlinear mixed effects modeling
NSAID	nonsteroidal anti-inflammatory drug
N_2	nitrogen
OTC	over-the-counter
O_2	Oxygen

pCO_2	partial pressure carbon dioxide
PD	pharmacodynamic
$P_{ET}CO_2$	end-tidal partial pressure carbon dioxide
PK	pharmacokinetic
pO_2	partial pressure oxygen
QA	quality assurance
QD	once daily
QTc	heart rate-corrected QT interval
ΔQTc	change-from-baseline in QTc
ΔΔQTc	placebo-adjusted change-from-baseline QTc
QTcF	heart rate-corrected QT interval using the Fridericia correction
RCF	relative centrifugal force
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SAP	statistical analysis plan
SD	standard deviation
SOP	standard operating procedure
SpO_2	oxygen saturation
$t_{1/2}$	terminal half-life
TEAE	treatment-emergent adverse event
T_{max}	time of C_{max}
VE55	minute ventilation at 55 mmHg partial pressure carbon dioxide

Appendix C – Protocol Revision History

PROTOCOL REVISION HISTORY			
Protocol Number	**Version**	**Effective Date**	**Summary of Changes**
SCR-012	1	21 June 2022	Developed initial protocol
SCR-012	1.1	21 July 2022	Updated protocol to reflect revised endpoints, assessments, and analyses method.

DocuSign

Certificate Of Completion

Envelope Id: 2B6DD0381EEF404EABE8B630AC2A9B25
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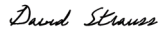
David Strauss david.strauss@fda.hhs.gov Sponsor Study Lead Security Level: Email, Account Authentication (Required)	 Signature Adoption: Pre-selected Style Signature ID: C37632FD-56B7-47FC-9B70-FAEE64302722 Using IP Address: 150.148.14.135 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 7/25/2022 2:37:47 PM Viewed: 7/27/2022 11:02:14 AM Signed: 7/27/2022 11:02:39 AM
Electronic Record and Signature Disclosure: Accepted: 10/25/2021 11:15:54 AM ID: 51a3664d-cfbd-4295-b370-e0bb8628e897		
Jan Matousek jan.matousek@spauldingclinical.com Prinicipal Investigator Security Level: Email, Account Authentication (Required)	 Signature Adoption: Pre-selected Style Signature ID: 4E887FB2-4D29-4750-80F9-DF0904DAE3DC Using IP Address: 12.11.209.41 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I have reviewed this document	Sent: 7/27/2022 11:02:45 AM Viewed: 7/27/2022 11:05:32 AM Signed: 7/27/2022 11:05:52 AM
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In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp
Jennifer Boganschneider jennifer.bogenschneider@spauldingclinical.com Security Level: Email, Account Authentication (Required) **Electronic Record and Signature Disclosure:** Not Offered via DocuSign	COPIED	Sent: 7/27/2022 11:02:45 AM

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	7/25/2022 2:37:47 PM
Certified Delivered	Security Checked	7/27/2022 11:05:32 AM
Signing Complete	Security Checked	7/27/2022 11:05:52 AM
Completed	Security Checked	7/27/2022 11:05:52 AM

Payment Events	Status	Timestamps

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