Study Title: Clinical Study to Investigate the Urinary Excretion of N-nitrosodimethylamine (NDMA) after Ranitidine Administration

Document Title: Clinical Study Protocol No. SCR-010

Document Date : 19 June 2020

NCT Number: NCT04397445

CLINICAL STUDY PROTOCOL

Clinical Study to Investigate the Urinary Excretion of N-nitrosodimethylamine (NDMA) after Ranitidine Administration

PROTOCOL NO. SCR-010

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Version of Protocol: 2.0

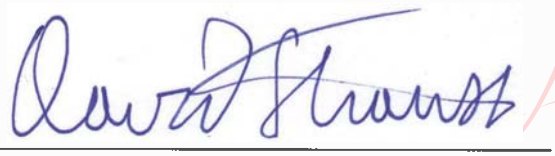
Date of Protocol: 19 June 2020

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).



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Date: 2020.06.22 15:00:21 -04'00'

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.

Colleen Nalepinski PA-C

Colleen Nalepinski MPAS, PA-C
Principal Investigator

22 JUN 2020

Date

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Protocol Synopsis

Protocol Number:	SCR-010
Title:	Clinical Study to Investigate the Urinary Excretion of N-nitrosodimethylamine (NDMA) after Ranitidine Administration
Investigators:	Principal Investigator: Colleen Nalepinski MPAS, PA-C Study Physician: Carlos Sanabria, MD
Study Phase:	1
Study Period:	The duration of study participation will be up to 10 days (excluding the screening period).
Study Site:	Spaulding Clinical Research Unit, West Bend, Wisconsin
Background and Motivation:	The U.S. Food and Drug Administration (FDA) has learned that some ranitidine products, including some products commonly known as the brand-name drug Zantac, contain a nitrosamine impurity called N-nitrosodimethylamine (NDMA) at low levels. NDMA is classified as a probable human carcinogen (a substance that could cause cancer) based on results from laboratory tests. NDMA is a known environmental contaminant and found in water and foods, including meats, dairy products, and vegetables (FDA Updates and Press Announcements on NDMA in Zantac (ranitidine)). The FDA has been investigating NDMA and other nitrosamine impurities in blood pressure and heart failure medicines called Angiotensin II Receptor Blockers (ARBs) since 2018. In the case of ARBs, the FDA has recommended numerous recalls as it discovered unacceptable levels of nitrosamines. FDA has found levels of NDMA in ranitidine active pharmaceutical ingredient and finished drugs that are similar to the levels you would expect to be exposed to if you ate common foods like grilled or smoked meats. FDA has requested removal of all ranitidine products from the market because some ranitidine products have NDMA levels above the acceptable limits (96 nanograms per day or 0.32 ppm for 300 mg per day of ranitidine) and that levels of NDMA in ranitidine may increase to unacceptable levels over time. However, FDA has not withdrawn approvals of ranitidine new drug applications and abbreviated new drug applications and if a company can show, through scientific data, that their ranitidine product is stable and the NDMA levels do not increase over time to unsafe levels, FDA may consider allowing that ranitidine product back on the U.S. market. The ranitidine that will be used in this study has been tested twice (months apart) and shown to have stable NDMA levels well below the acceptable daily limit. Of note, the risk of NDMA with ranitidine is only relevant with prolonged chronic

administration as at the acceptable limit, there is approximately a 1 in 100,000 chance of cancer after 70 years of exposure to that level.

FDA has also conducted tests that simulate the potential formation of NDMA from ranitidine after it has been exposed to acid in the stomach with a normal diet. Results of these tests indicate that NDMA is not formed in typical stomach conditions. Similarly, if ranitidine is exposed to a simulated small intestinal fluid, NDMA is not formed. Other in vitro experiments suggest a combination of nitrites, such as found in processed meats, and an acidic environment potentiate formation of NDMA. For that reason, prior to requesting removal of ranitidine products from the market, the FDA had advised consumers who wished to continue taking these medications to consider limiting consumption of nitrite-containing foods.

Separately, a previous study (Zeng and Mitch, 2016) in 10 healthy volunteers showed that single dose administration of ranitidine 150 mg was associated with ~400-fold increase in urinary NDMA excreted over 24 hours. This level of increase is substantially greater than would be expected from the laboratory testing conducted by FDA. Further evaluation is necessary to determine if and how much NDMA is produced from ranitidine in the human body and whether nitrite-containing foods may potentiate formation of NDMA in vivo.

Ranitidine

Ranitidine is an over-the-counter (OTC) and prescription drug. Ranitidine is an histamine-2 (H_2) blocker, which decreases the amount of acid secreted by the stomach. Over-the-counter ranitidine is approved to prevent and relieve heartburn associated with acid ingestion and sour stomach and the approved dosage regimen is up to 150 mg twice a day. Prescription ranitidine is approved for multiple indications, including treatment and prevention of ulcers of the stomach and intestines and treatment of gastroesophageal reflux disease and the approved dosage regimen is up to 150 mg twice a day or 300 mg once a day.

Summary

This clinical study will use a prescription dose of ranitidine (300 mg) to test whether there is increased urinary NDMA excretion levels 24-hours after ranitidine administration in comparison to placebo when subjects are administered a low nitrite/NDMA meal and when subjects are administered a high nitrite/NDMA meal. This will be accomplished through a 4-period crossover study:

A. Ranitidine + low nitrite/NDMA meal;
B. Placebo + low nitrite/NDMA meal;
C. Ranitidine + high nitrite/NDMA meal;
D. Placebo + high nitrite/NDMA meal.

Objectives and Endpoints:

The objectives of this study are:

Primary Objective

1. To evaluate 24-hour urinary excretion of NDMA after oral administration of ranitidine compared to placebo

Exploratory Objectives

1. To evaluate plasma ranitidine, NDMA, and dimethylamine (DMA) after oral administration of ranitidine compared to placebo
2. To evaluate urinary excretion amounts over 24-hours of ranitidine and DMA after oral administration of ranitidine compared to placebo
3. To evaluate 24-hour urinary excretion and plasma concentration of NDMA and DMA with administration of high nitrite/NDMA meals compared to low nitrite/NDMA meals

The endpoints for this study are:

Primary Endpoint

1. 24-hour urinary NDMA excretion

Exploratory Endpoints

1. $AUC_{0\text{-}inf}$ of plasma ranitidine, NDMA and DMA
2. Cumulative ranitidine and DMA amount excreted in urine over 24 hours after ranitidine administration

Study Design:

This is a randomized, placebo-controlled, single-dose, 4-period crossover study with 18 healthy subjects with the following design:

Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Check-in	Washout	Period 1	Washout	Period 2	Washout	Period 3	Washout	Period 4	Checkout

The following 4 treatments will be administered over the 4 study periods. Each subject will be randomized to 1 of 4 treatment sequences (i.e., ABCD, ABDC, BACD, or BADC). FDA will prepare the randomization schedule. The treatments consist of oral administration of either a single dose of ranitidine (300 mg) or placebo administered at time 0 hr. All subjects will be provided low nitrite/NDMA meals for the first two periods of the study and high nitrite/NDMA meals for the last two periods of the study. This ordering of meals will allow purchasing a single lot of perishable items for different meals and to simplify meal preparation and serving at the study site.

Treatment Code	Treatment
A	Ranitidine + low nitrite/NDMA meal
B	Placebo + low nitrite/NDMA meal
C	Ranitidine + high nitrite/NDMA meal

D	Placebo + high nitrite/NDMA meal

Subjects will report to the study site for screening from Days -28 to -3 and then will return to the site on Day -2 for baseline assessments. Subjects will receive three standardized meals per day starting on Day -1. Subjects will be served meals from a pre-specified menu for check-in and for all washout and treatment days. Subjects will be instructed to finish all their meals within 25 minutes with no leftovers. Subjects will only consume foods served to them at planned meal and snack times.

Two different full day menus of low nitrite/NDMA and high nitrite/NDMA meals have been developed. Additional details regarding the meals will be specified in the Meal Preparation Plan. Meals will be identical for treatments A and B (low nitrite/NDMA meal) of the study and a separate set of identical low nitrite/NDMA meals will be served on the washout days prior to treatment. Likewise, meals will be identical for treatments C and D (high nitrite/NDMA meal) and a separate set of identical high nitrite/NDMA meals will be served on the washout days prior to treatment. The last meal on Day -1, Day 2, Day 4, and Day 6 should be administered at approximately 6 PM to permit at least 12-hour fasting prior to dosing. Subjects will be provided with distilled water to drink throughout the study.

On study treatment days, the first meal will be provided at the time of dosing. Subjects will be instructed to swallow the medication with approximately 250 mL of room temperature distilled water and begin eating within two minutes after dosing. Subjects are required to eat each meal in its entirety during the study. If the meal is not finished, the reason should be recorded, along with what was not eaten, and a picture of the remaining food should be taken.

Prior to and following study drug or placebo administration on Day 1, subjects will undergo assessments as described in the Schedule of Events (Table 8-1). There will be one day of washout between periods. Participants will be confined in the study clinic from Day -2 until the morning of Day 8.

During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is eligible for the study. Subjects will be shown the low and high nitrite/NDMA menus and informed that each meal in the menu must be finished in its entirety. The subject must agree to consume all planned meals in order to be eligible to participate. 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, serum 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 3 days before Day 1.

Results of all screening tests will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility. For subjects that are eligible, a molecular diagnostic test for SARS-CoV2 will be performed just before (e.g. ~2 days before) or at check-in depending on the testing turnaround to enable results prior to admission.

At check-in (Day -2), eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented. Vital sign measurements and a 12-lead electrocardiogram (ECG) will be performed. Clinical laboratory, drug, alcohol, and pregnancy tests (for females) will be performed. An intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed).

Urine and blood samples for pharmacokinetic assessments will occur before or after drug or placebo administration at the following timepoints:

- Urine samples will be collected using separate collection containers over 24 h. Collection times will occur at 0 (pre-dose), 3, 6, 9, 12, 15, and 24 h. Subjects will be instructed to void their bladder at each collection time and total weight of the sample will be recorded. If a subject must void their bladder at an unscheduled time (highly discouraged), the unscheduled voids will be collected, and total weight of the unscheduled voiding will be recorded. The unscheduled voiding sample will be treated, analytically analyzed, and reported as part of scheduled sample collection for determining cumulative amounts of NDMA, ranitidine, and DMA excreted over 24 h.
- Plasma samples will be collected at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 11, 14, and 24 h post-dose.

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. Meals (timing and components), activity levels, and general conditions in the study clinic will be standardized to the extent possible on the treatment days.

Subject Population:

Approximately 18 healthy subjects are planned for enrollment. Every effort will be made to maintain an approximate 50:50 male-to-female sex distribution. Up to 4 subjects may be qualified as replacements. Thus, a maximum of 22 subjects may be exposed to study drugs and procedures during the study.

Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the Institutional Review Board (IRB) before

telephone screening. Subjects will be offered payment for Screening and participation in the study, but no special incentives are offered.

Study and Reference Drugs, Dosage, and Route of Administration:

Ranitidine 300 mg oral tablets and oral placebo will be supplied. On Days 1, 3, 5, and 7 at 0 h, subjects will receive one dose of oral ranitidine or oral placebo followed by a meal starting within 2 minutes of dosing.

Inclusion Criteria:

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

1. Subject is willing and able to sign 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 32 kg/m^2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 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 (Day -2).
5. Female subjects must be of non-childbearing potential or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before Check in (Day -2) 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 -2) until at least 1 month after the end of the study.
6. 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 antacids or proton pump inhibitors within 14 days of screening (interferes with *H. pylori* testing).
2. Subject has used any prescription or nonprescription drugs (including antacids, proton pump inhibitors, 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.

3. 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.
4. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks of Screening.
5. 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. Subjects must also refrain from using mouthwash from check-in until check-out.
6. 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 higher risk for coronavirus disease of 2019 (COVID-19) complications; per current Center for Disease Control and Prevention (CDC) recommendations this includes:
    - People with chronic lung disease or moderate to severe asthma
    - People who have serious heart conditions
    - People who are immunocompromised
    - Many conditions can cause a person to be immunocompromised, including cancer treatment, smoking, bone marrow or organ transplantation, immune deficiencies, poorly controlled HIV, and prolonged use of corticosteroids and other immune weakening medications
    - People with severe obesity (body mass index [BMI] of 40 or higher)
    - People with diabetes
    - People with chronic kidney disease undergoing dialysis
    - People with liver disease
7. Subject has any signs or symptoms 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.

8. Subject tests positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by a molecular diagnostic test performed prior to admission.
9. Subject has known or suspected allergies or sensitivities to the study drug.
10. Subject has clinical laboratory test results (hematology, serum chemistry and urinalysis) at Screening or Check-In that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator.
11. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.
12. Subject has a history of H. pylori infection or ulcer disease or has a positive breath test for H. pylori at screening.
13. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.
14. Female subjects are pregnant or lactating before enrollment in the study.
15. Subject is not willing to eat all of every meal that will be served during the study.

Sample Collection

The following samples should be collected and processed as follows. Specific timepoints can be found in the Schedule of Events:

1. The blood samples (8 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 revolutions per minute, at 4°C, by a study team member.

   The plasma will be separated using a disposable plastic pipette and will be 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 until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

2. Prior to sample collection, prepare three separate storage containers (Aliquot A, B, and C) for sample collection. Add 240 µL of 5M sodium hydroxide to the sample containers for Aliquot A and Aliquot B and placed on ice. Place the storage container for Aliquot C on ice without adding sodium hydroxide. At specific time points, urine will be collected using a separate container. Subjects will be instructed to void their

bladder at each collection time. Urine will be collected in a tared container and immediately after collection (i.e., within 5 min), the sample should be weighed, and the weight recorded. Transfer three 40 mL aliquots of the urine sample to each of the storage containers. After transferring the urine in the prepared containers, mix the urine and sodium hydroxide (Aliquots A and B) by vigorously shaking to ensure mixing. All aliquots should be kept in an ice bath during these steps, and the entire process up through this point should be completed within 15 min of sample collection. All samples should be moved to the freezer within the next 15 min and stored at -80oC. All steps must be completed within 30-min from the time of voiding. If the volume is less than 60 mL, the sample should be divided into three equal aliquots of the collected volume. The volume a sodium hydroxide added should be decreased proportionally to the final aliquot volume. If a subject must void their bladder at an unscheduled time (highly discouraged), the unscheduled voids will be collected and must be handled in a similar manner as scheduled void sample.

Pharmacokinetic Assessments:

Samples will be collected as described above and in the Schedule of Events for determination of NDMA, DMA, and ranitidine in urine or blood. The following PK parameters will be determined for each subject for NDMA, DMA, and ranitidine:

Urine

- Cumulative amount excreted in urine over 24 h (Ae_{0-24})
- Total fraction of dose excreted in urine (Total Fe, ranitidine only)
- Renal clearance (ranitidine) (CL_R)

Plasma

- AUC from time 0 extrapolated to infinity (AUC_{0-inf})
- AUC from time 0 to the sampling time corresponding to the last quantifiable concentration (C_{last}) (AUC_{0-t})
- Maximum concentration (observed peak drug concentration) (C_{max})
- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (Kel)
- Terminal half-life ($t_{1/2}$)

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:

Eighteen healthy subjects are planned to be enrolled. Fourteen subjects are required to detect an increase in cumulative NDMA excreted in urine over 24-hours following ranitidine administration compared to placebo assuming a 2-fold increase in NDMA with ranitidine administration. Using a log-transformed paired comparison of cumulated amounts and 100% coefficient of variability, there is greater than 90% power at a one-sided significance level. Up to 4 subjects may be qualified as replacements if needed.

Statistical Methods:

All data will be presented in data listings. Data from subjects excluded from the PK 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 PK population will include all subjects who receive study drug and have at least one on-study sample. The analysis population will include all subjects in the PK population with calculated PK parameters from at least two study periods of the same meal type (i.e., low nitrite/NDMA or high nitrite/NDMA).

Primary Analysis

Pharmacokinetics: Cumulative amount of NDMA excreted in urine over 24-hours is the primary PK parameter of interest. This will be determined by calculating cumulative amount excreted during specified intervals, and summarizing totals over a 24-h period. Similar approaches will be utilized for determining cumulative amount of DMA and ranitidine excreted in urine over 24-h.

Area under the curve and additional PK parameters from ranitidine, DMA, and NDMA plasma concentrations will be determined for each subject using non-compartmental methods. All parameters will be reported with standard descriptive statistics including the geometric mean and coefficient of variation. Calculation of PK parameters will be performed using actual sampling times.

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: 19 June 2020

List of Abbreviations

Abbreviation	Definition
AE	adverse event
Ae_{0-24}	cumulative amount excreted in urine over 24-hours
ANOVA	analysis of variance
ARB	angiotensin II receptor blocker
AUC_{0-inf}	area under the concentration-time curve from 0 to infinity
AUC_{0-t}	area under the concentration-time curve from 0 to last quantifiable concentration
CFR	Code of Federal Regulations
CI	confidence interval
CL	clearance
C_{last}	last quantifiable concentration
CL_R	renal clearance
C_{max}	maximum observed concentration
COVID-19	coronavirus disease of 2019
ECG	electrocardiogram
eCRF	electronic case report form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
H2 blocker	histamine-2 blocker
HBsAg	hepatitis B surface antigen
HepC	hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
ICH	International Council for Harmonisation
IRB	Institutional Review Board
IV	intravenous
Kel	elimination rate constant
Kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
Mg	milligram
NDMA	N-nitrosodimethylamine
NSAID	nonsteroidal anti-inflammatory drug
OTC	over-the-counter
PK	pharmacokinetic
Ppm	parts per million
QA	quality assurance

SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
$t_{1/2}$	terminal half-life
TEAE	treatment-emergent adverse event
T_{max}	time of C_{max}
Total Fe	total fraction of dose excreted in urine

1. Introduction

The U.S. Food and Drug Administration (FDA) has learned that some ranitidine medicines, including some products commonly known as the brand-name drug Zantac, contain a nitrosamine impurity called N-nitrosodimethylamine (NDMA) at low levels. NDMA is classified as a probable human carcinogen (a substance that could cause cancer) based on results from laboratory tests. NDMA is a known environmental contaminant and found in water and foods, including meats, dairy products, and vegetables (FDA Updates and Press Announcements on NDMA in Zantac (ranitidine)).

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FDA has found levels of NDMA in ranitidine active pharmaceutical ingredient and finished drugs that are similar to the levels you would expect to be exposed to if you ate common foods like grilled or smoked meats. FDA has requested removal of all ranitidine products from the market because some ranitidine products have NDMA levels above the acceptable limits (96 nanograms per day or 0.32 ppm for 300 mg per day of ranitidine) and that levels of NDMA in ranitidine may increase to unacceptable levels over time. However, FDA has not withdrawn approvals of ranitidine new drug applications and abbreviated new drug applications and if a company can show, through scientific data, that their ranitidine product is stable and the NDMA levels do not increase over time to unsafe levels, FDA may consider allowing that ranitidine product back on the U.S. market. The ranitidine that will be used in this study has been tested twice (months apart) and shown to have stable NDMA levels well below the acceptable daily limit. Of note, the risk of NDMA with ranitidine is only relevant with prolonged chronic administration as at the acceptable limit, there is approximately a 1 in 100,000 chance of cancer after 70 years of exposure to that level.

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Summary

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B. Placebo + low nitrite/NDMA meal;
C. Ranitidine + high nitrite/NDMA meal;
D. Placebo + high nitrite/NDMA meal.

2. Study Objectives

2.1. Primary Objective

The primary objective of this study is:

1. To evaluate 24-hour urinary excretion of NDMA after oral administration of ranitidine compared to placebo

2.2. Exploratory Objective

The exploratory objectives of this study are:

1. To evaluate plasma ranitidine, NDMA and dimethylamine (DMA) after oral administration of ranitidine compared to placebo
2. To evaluate urinary excretion amounts over 24-hours of ranitidine and DMA after oral administration of ranitidine compared to placebo
3. To evaluate 24-hour urinary excretion and plasma concentration of NDMA and DMA with administration of high nitrite/NDMA meals compared to low nitrite/NDMA meals

3. Study Endpoints

3.1. Primary Endpoint

The primary endpoint of this study is:

1. 24-hour urinary NDMA excretion

3.2. Exploratory Endpoints

The exploratory endpoints of this study are:

1. $AUC_{0\text{-}inf}$ of plasma ranitidine, NDMA and DMA
2. Cumulative ranitidine and DMA amount excreted in urine over 24 h after ranitidine administration

4. Investigational Plan

4.1. Study Design

This is a randomized, placebo-controlled, single-dose, 4-period crossover study in 18 healthy subjects. Healthy subjects will be randomized to one of 4 treatment sequences (i.e., ABCD, ABDC, BACD, or BADC). Four treatments shown in Table 4-1 will be given to each subject over the four study periods (see Table 4-2). All subjects will be provided low nitrite/NDMA meals for the first two periods of the study and high nitrite/NDMA meals for the last two periods of the study. This ordering of meals is proposed to allow purchasing a single lot of perishable items for different meals and to simplify meal preparation and serving at the study site. These sources of variability are expected to exceed variability from sequence effects. Furthermore, the proposed randomization sequences only impact the exploratory objective comparing NDMA and DMA exposure resulting from different meals.

Table 4-1: Treatments

Treatment Code	Treatment
A	Ranitidine + low nitrite/NDMA meal
B	Placebo + low nitrite/NDMA meal
C	Ranitidine + high nitrite/NDMA meal
D	Placebo + high nitrite/NDMA meal

Table 4-2: Study Schedule

Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Check-in	Washout	Period 1	Washout	Period 2	Washout	Period 3	Washout	Period 4	Checkout

At the study clinic (Spaulding Clinical Research unit in West Bend, Wisconsin), subjects will report to the study site for screening from days -28 to -3. During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. Subjects will be shown the low nitrite/NDMA and high nitrite/NDMA menus and informed that each meal in the menu must be finished in its entirety. The subject must agree to consume all planned meals in order to be eligible to participate. 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, vital signs and 12-lead ECG will be performed by a study team member. Clinical laboratory tests (hematology, serum chemistry, urinalysis) along with urine drug and alcohol screen, HIV test, hepatitis test, *H. pylori* breath test 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 3 days before Day 1.

Results of all screening tests will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility. For subjects that are eligible, a molecular diagnostic test for SARS-CoV2 will be performed just before (e.g. ~2 days before) or at check-in depending on the testing turnaround to enable results prior to admission. At check-in, eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented, vital sign measurements and a 12-lead electrocardiogram (ECG) will be performed, clinical laboratory, drug and alcohol, and pregnancy tests (for females) will be performed, an intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed), study drug will be administered, and blood samples will be collected per protocol.

Subjects will enter the study clinic site on Day -2 for baseline assessments. Starting on Day -1, subjects will receive three standardized meals per day that will be specified in a separate study meal plan document. Subjects will be instructed to finish all their meals in 25-min with no leftovers. Subjects will only consume foods served to them at planned meal and snack times. Two different full day menus of low nitrite/NDMA and high nitrite/NDMA meals have been developed. Meals will be identical for treatments A and B (low nitrite/NDMA meal) of the study and a separate set of identical low nitrite/NDMA meals will be served on the washout days prior to treatment. Likewise, meals will be identical for treatments C and D (high nitrite/NDMA meal) and a separate set of identical high nitrite/NDMA meals will be served on the washout days prior to treatment. The last meal on Day -1, Day 2, Day 4, and Day 6 will be administered at a time to enable at least 12-h fasting prior to dosing. Subjects will be provided with distilled water to drink throughout the study. Two servings of each Day -1 and Day 1 meal from the low nitrite/NDMA

and high nitrate/NDMA menus may be processed for analysis of nitrite, nitrate, and NDMA content.

On study treatment days, the first meal will be provided at the time of dosing. Subjects will be instructed to swallow the medication with approximately 250 mL of room temperature distilled water and begin eating breakfast within 2-min after dosing. Subjects are required to eat all of each meal during the study. If the meal is not finished, the reason should be recorded, along with what was not eaten, and a picture of the remaining food should be taken.

FDA will prepare the randomization schedule. Either ranitidine and placebo will be administered orally on Day 1, 3, 5, and 7 at 0 h. Prior to and following study drug or placebo administration, subjects will undergo assessments as described in the Schedule of Events (Table 8-1). There will be one day of washout between periods. Participants will be confined in the study clinic from Day -2 until the morning of Day 8.

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 (see Table 8-1).

4.1.1 Dosing Schedule

At the beginning of each period (i.e., Day 1, 3, 5, and 7 at 0 h), subjects will receive one of the following treatments:

- Oral ranitidine 300 mg
- Oral placebo

4.1.2 Risk/Benefit

Subjects will be informed that participation in a human PK 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 if and how much NDMA is produced from ranitidine in the human body. 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 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 ranitidine 300 mg will be provided to and discussed with the subjects:

- Headache, sometimes severe, is the most common adverse effect. The following additional adverse events have been reported in patients taking ranitidine, but whether they were caused by ranitidine has been unclear in many cases:
    - Neurological effects: Rarely malaise (general discomfort), dizziness, somnolence (sleepiness), insomnia (difficulty sleeping), vertigo (dizziness). Rare cases of reversible mental confusion, agitation, depression, hallucinations mainly in severely ill elderly patients, reversible blurred vision, or reversible involuntary motor disturbances.
    - Heart effects: Rare reports of abnormal heart rhythms.
    - Abdominal effects: Constipation, diarrhea, nausea/vomiting and abdominal discomfort/pain. Occasional reports of hepatitis (liver inflammation). Rare cases of liver failure or pancreatitis (inflammation of pancreas).
    - Musculoskeletal effects: Rare reports of arthralgias (joint pain) and myalgias (muscle aches).
    - Blood/vessel effects: Leukopenia, granulocytopenia and thrombocytopenia (decreased blood cell counts) in a few patients. Rare cases of vasculitis (blood vessel inflammation), agranulocytosis or pancytopenia (more severe decreased blood counts), and exceedingly rare cases of acquired immune hemolytic anemia (low red blood cell count).
    - Sexual effects: Controlled studies have shown no hormone effects, however, occasional cases of impotence (erectile dysfunction), and loss of libido (sex drive) in males, but the rates did not differ from that in the general population. Rare cases of breast symptoms and conditions, including galactorrhea (milky nipple discharge) and gynecomastia (enlarged breast tissue).
    - Skin/hair effects: Rash, including rare cases of erythema multiforme (skin or mouth lesions). Rare cases of alopecia (hair loss).
    - Lung effects: Potential increased risk of pneumonia (lung infection).
    - Other: Rare cases of hypersensitivity reactions (exaggerated immune response that can cause trouble breathing, fever, rash or increased blood counts), anaphylaxis (severe allergic reaction), angioneurotic edema (swelling under the skin), acute interstitial nephritis (kidney inflammation), and small increases in serum creatinine (effect on kidneys).

In addition, subjects will be informed the purpose of this study is to evaluate whether ranitidine administration results in increased NDMA levels in urine and plasma. Subjects will be informed that NDMA is classified as a probable known carcinogen, however the ranitidine that subjects take in this study has been tested and shown to be well below the acceptable limits and these

levels are stable on repeated testing. At the acceptable limit, there is approximately a 1 in 100,000 chance of cancer after 70 years of exposure to that level.

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 woman 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.

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 may eat only meals and snacks that are provided during periods of their stay in the study clinic, and that they must consume all of each meal that is served at a reasonable pace (within 25 minutes). The required meals will be presented to the subjects during the screening process.

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 that will limit the risk of COVID-19. Precautions will be documented in a COVID-19 risk management plan. Currently, this includes phone screening to prevent symptomatic participants from entering the clinic; triage of all potential study subjects entering the building at screening and check-in for potential contacts with COVID-19, signs and symptoms, temperature monitoring and potential serology screening for severe acute respiratory syndrome corona virus 2 (SARS CoV-2); SARS CoV-2 molecular testing just prior to or at check-in for admission to the study floor; all study participants and staff wearing masks except when in a private room or for a limited time for a study procedure (e.g. study drug administration); staff wearing personal protective equipment, social distancing during screening and in-house stays including 1 subject per room for overnight stays; extra hand sanitation stations with hand washing and sanitation policies per CDC recommendations; closing common areas and serving food at subjects' room resulting in subjects spending most of their time in their rooms with the exception of specified times for walking in the halls; daily temperature screening; and separate staff for confined vs. not-confined participants whenever possible. 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. 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.

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.

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 18 healthy subjects are planned for enrollment. Up to 4 subjects may be qualified as replacements. Thus, a maximum of 22 subjects may be exposed to study drugs and procedures during the study. 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 of 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 32 kg/m^2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 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 (Day -2).
5. Female subjects must be of non-childbearing potential or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before Check in (Day -2) 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 -2) until at least 1 month after the end of the study.
6. 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 antacids or proton pump inhibitors within 14 days of screening (interferes with *H. pylori* testing).
2. Subject has used any prescription or nonprescription drugs (including antacids, proton pump inhibitors, 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.
3. 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
4. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks of Screening
5. 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. Subjects must also refrain from using mouthwash from check-in until check-out.
6. 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 higher risk for coronavirus disease of 2019 (COVID-19) complications; per current Center for Disease Control and Prevention recommendations this includes:
    - People with chronic lung disease or moderate to severe asthma
    - People who have serious heart conditions
    - People who are immunocompromised
    - Many conditions can cause a person to be immunocompromised, including cancer treatment, smoking, bone marrow or organ transplantation, immune deficiencies, poorly controlled HIV or AIDS, and prolonged use of corticosteroids and other immune weakening medications
    - People with severe obesity (body mass index [BMI] of 40 or higher)
    - People with diabetes
    - People with chronic kidney disease undergoing dialysis
    - People with liver disease

7. Subject has any signs or symptoms 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.
8. Subject tests positive for SARS-CoV-2 by a molecular diagnostic test performed prior to admission.
9. Subject has known or suspected allergies or sensitivities to the study drug.
10. Subject has clinical laboratory test results (hematology, serum chemistry and urinalysis) at Screening or Check-In that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator.
11. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.
12. Subject has a history of *H. pylori* infection or ulcer disease or has a positive breath test for *H. pylori* at screening.
13. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.
14. Female subjects are pregnant or lactating before enrollment in the study.
15. Subject is not willing to eat all of every meal that will be served during the study.

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 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 Subjects

Approximately 18 healthy subjects are planned for enrollment and will be randomized to 1 of 4 treatment sequences. Up to 4 subjects may be qualified as replacements. Thus, a maximum of 22 subjects will be exposed to study drugs and procedures during the study.

If a subject vomits after dosing, a blood sample will be collected and, if the subject vomits immediately after dosing, the sponsor will be consulted regarding a decision to continue the subject in the study. Under no circumstances will a dose of the study drug be repeated.

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 Day –3):

- 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 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, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a complete physical examination
- Perform *H. pylori* breath test

4.6.4 Study Periods

This is a randomized, placebo-controlled, single-dose, 4-period crossover study with 18 healthy subjects (see Table 4-1 and Table 4-2). Healthy subjects will be randomized to 1 of 4 treatment sequences (i.e., ABCD, ABDC, BACD, or BADC). Subject will be kept in confinement from Day -2 (Check-in) through the morning of Day 8 (Check-out).

4.6.4.1 Check-in

The following procedures and assessments will be performed at Check-in (Day -2):

- Perform/review results from SARS-CoV-2 molecular test (may be performed ~2 days before check-in to allow time for results)
- 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 a serum pregnancy test (female subjects only)
- Perform FSH measuring (postmenopausal [i.e., without menses for two years] female subjects only)
- Admit subject to the study clinic
- Randomization (after completion of check-in procedures on Day -2 or just before dosing on Day 1)

- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Measure height, weight, and calculate body mass index
- Perform a safety 12-lead ECG
- Perform a comprehensive physical examination

4.6.4.2 Treatment

The following procedures and assessments will be performed during the treatment period according to the Schedules of Events (Table 8-1):

- Monitor for AEs
- Record concomitant medications
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Administer study drug according to the randomization schedule following all other pre-dose examinations and specimen collection
- Insert IV catheter for blood sampling (if necessary)
- Collect blood samples (8 mL for each sample)
- Collect a urine sample in a specimen collection container. Record weight of the entire sample and collect up to 120 mL of the sample for processing

4.6.5 Discharge (or Early Termination)

The following procedures and assessments will be performed before the subject is discharged from the study (Day 8) or at early termination according to Table 8-1:

- Perform a serum pregnancy test (female subjects only)
- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a complete physical examination

- Measure height, weight, and calculate body mass index
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Collect a single blood sample (8 mL)
- Collect a urine sample in a specimen collection container. Record weight of the entire sample and collect up to 120 mL of the sample for processing
- Remove IV catheter (if applicable)
- 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 (8 mL for each sample) for determination of ranitidine and NDMA will be collected at the following time points on Days 1, 3, 5, and 7: 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 11, 14, and 24 h.

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. 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.

Urine samples will be collected at the following time points on Days 1, 3, 5, and 7 for determination of cumulative amount of ranitidine and NDMA excreted in urine over 24-h: 0 (pre-dose), 3, 6, 9, 12, 15, and 24 h. Subjects will be instructed void their bladder at each collection time and total weight of the sample will be recorded. If a subject must void their bladder at an unscheduled time (highly discouraged), the unscheduled voids will be collected, and total weight of the unscheduled voiding will be recorded. The unscheduled voiding sample will be treated, analytically analyzed, and reported as part of scheduled sample collection for determining cumulative amounts of NDMA, ranitidine, and DMA excreted over 24 h.

4.7.1.2 Blood and Urine Specimen Handling

The blood samples (8 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 revolutions per minute, 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). Plasma samples

will be appropriately labeled and stored frozen at –70°C or below until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

Prior to urine sample collection, three separate storage containers (Aliquot A, B, and C) for sample collection will be prepared. Aliquot A and Aliquot B will have 240 µL of 5M sodium hydroxide added to the sample containers and placed on ice; Aliquot C will be place on ice without adding sodium hydroxide. Urine samples will be collected in a specimen collection container. Subjects will be instructed to void their bladder into the specimen collection container at each collection time. If a subject must void their bladder at an unscheduled time (highly discouraged), the unscheduled voids will be collected and must be handled in a similar manner as scheduled collections. Urine should be collected in a tared container and immediately after collection (i.e., within 5 min), the sample should be weighed, and the weight recorded. After weighing, three 40 mL aliquots of the urine sample should be transferred to each of the storage containers. After transferring the urine in the prepared containers, the urine and sodium hydroxide (Aliquots A and B) should be shaken vigorously to ensure mixing. All aliquots should be kept in an ice bath during these steps, and the entire process up through this point should be completed within 15 min of sample collection. All samples should be moved to the freezer within the next 15 min and stored at -80°C. All steps must be completed within 30-min from the time of sample collection. If the volume is less than 60 mL, the sample should be divided into three equal aliquots of the collected volume. The volume a sodium hydroxide added should be decreased proportionally to the final aliquot volume.

Additional details can be found in the Urine Sample Collection SOP.

4.7.1.3 Pharmacokinetic Parameters

The following PK parameters will be determined for each subject:

- Urine
    - Cumulative amount excreted in urine over 24 h (Ae_{0-24})
    - Total fraction of dose excreted in urine (Total Fe)
    - Renal clearance (ranitidine) (CL_R)
- Plasma
    - AUC from time 0 extrapolated to infinity (AUC_{0-inf})
    - AUC from time 0 to the sampling time corresponding to the last quantifiable concentration (C_{last}) (AUC_{0-t})
    - Maximum concentration (observed peak drug concentration) (C_{max})
    - Time at which C_{max} occurs (T_{max})
    - Elimination rate constant (Kel)

- Terminal half-life ($t_{1/2}$)

4.7.2 Meals Assessments

All meals prepared for subjects will utilize foods shown in the literature to have low nitrite/NDMA or high nitrite/NDMA content. Two servings of each low nitrite/NDMA meal and each high nitrate/NDMA meal from Period 1 and Period 3 of the study, respectively, will be frozen at -60°C or below to enable potential analysis of nitrite, NDMA and nitrate content.

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 Schedules of Events (Table 8-1). 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 Schedules of Events (Table 8-1) and will be collected in accordance with acceptable laboratory procedures. Clinical laboratory testing will be performed by Spaulding Clinical Research or Quest Diagnostics. 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 and 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**	**Other**
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)	Drug screen including: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone	*H. pylori* breath test SARS-CoV2 molecular 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 Schedules of Events (Table 8-1). The subject should be in a supine position, if possible, for a minimum of 5 minutes before vital signs are measured.

4.7.3.4 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.

4.7.3.5 Physical Examinations

A complete physical examination will be performed at the time points presented in the Schedules of Events (Table 8-1).

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 Treatments Administered

Subjects will be randomized to 1 of 4 treatment sequences (i.e., ABCD, ABDC, BACD, or BADC) and will receive the first treatment on Day 1 at 0 h and the subsequent treatments on Days 3, 5, and 7 at 0 h:

Treatment Code	Treatment
A	Ranitidine + low nitrite/NDMA meal
B	Placebo + low nitrite/NDMA meal
C	Ranitidine + high nitrite/NDMA meal
D	Placebo + high nitrite/NDMA meal

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.

4.8.2 Dose Selection

4.8.2.1 Ranitidine

A ranitidine dose of 300 mg was selected as it is the maximum FDA-approved dose that a patient can take per dose (i.e., 300 mg once daily for the treatment of duodenal and/or gastric ulcer). Of note, for OTC use to relieve heartburn, doses up to 300 mg/day (i.e., 150 mg twice daily) are recommended.

4.8.3 Method of Assigning Subjects to Treatment Sequence

4.8.3.1 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 -2 or just before meal administration on Day -1. Approximately 18 healthy subjects are planned for enrollment. Up to 4 subjects may be qualified as replacements. Thus, a maximum of 22 subjects may be exposed to study drugs and procedures during the study. Unique subject numbers will be used in sequential order based on each subject's order of qualification.

Enrolled subjects will be randomly assigned to 1 of 4 treatment sequences (e.g., ABCD, ABDC, BACD, or BADC). The treatment groups are presented in Table 4-4.

Table 4-4: Study Treatment Groups

Subjects (n)	Treatment Group	Drug
18	A	Ranitidine + low nitrite/NDMA meal
	B	Placebo + low nitrite/NDMA meal
	C	Ranitidine + high nitrite/NDMA meal
	D	Placebo + high nitrite/NDMA meal

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.

4.8.4 Identity of Study Drugs

Ranitidine is a competitive, reversible inhibitor of the action of histamine at the histamine H_2-receptor, including receptors on the gastric cells (e.g., H_2-receptor antagonist). Over-the-counter ranitidine is approved to prevent and relieve heartburn associated with acid ingestion and sour stomach. Prescription ranitidine is approved for multiple indications, including treatment and prevention of ulcers of the stomach and intestines and treatment of gastroesophageal reflux disease. Ranitidine has a molecular weight of 350.87 grams and a molecular formula of $C_{13}H_{22}N_4O_3S{\bullet}HCl$ (Ranitidine Package Insert [Glenmark Pharmaceuticals Inc.], 2018).

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

4.8.5 Management of Clinical Supplies

4.8.5.1 Study Drug Packaging and Storage

The active study drugs will be obtained from commercial sources. For ranitidine, FDA will evaluate the specific lot to determine that is has NDMA levels below the acceptable daily intake limit (0.096 micrograms or 0.32 ppm for ranitidine). Storage instructions for the active study drugs are as follows:

- Ranitidine tablets should be stored between 15°C to 30°C (59°F to 86°F). Store in a dry place. Avoid excessive heat (temperatures above 40 °C [104°F]) and protect from light.
- Placebo should be stored at controlled room temperature (15°C to 30°C; 59°F to 86°F) and protected from light and moisture.

4.8.5.2 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.

4.8.6 Blinding

The study will be double-blind, and the blind will be maintained through a randomization schedule held by the dispensing pharmacist. In addition, subjects will be blindfolded during study drug administration. 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.

4.8.6.1 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.

4.8.7 Treatment Compliance

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 4.5.2.

4.8.8 Prior and Concomitant Medications

Subjects are prohibited from using any prescription or nonprescription drugs (including antacids, proton pump inhibitors, aspirin or non-steroidal anti-inflammatory drugs [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. Subject are also prohibited from using antacids or proton pump inhibitors within 14 days of screening (interferes with H. pylori testing). 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.

4.8.9 Subject Restrictions

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) throughout the duration of the study.

Subjects must be willing to comply with study rules, including the meal schedule; 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.

All subjects will fast overnight for a minimum of 8 h (no food or fluid except water) before blood collection for clinical laboratory testing and for 12 h before study drug/placebo dosing. Standardized meals will be served at consistent times relative to dosing, and no food or fluids will be served containing caffeine. Outside of meal times, the subjects will only be allowed to intake distilled water, which will be available ad libitum.

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

- Subjects must always 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 1 subject per room for overnight stays and having common areas closed. Food will be served at subjects' rooms with subjects sitting at their doorway to eat. Subjects will spend most of their time in their rooms except for specified times for walking in the halls (with masks).
- 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.

If new information becomes available, there could be other precautions that lead to additional restrictions.

4.9. Statistical Methods

4.9.1 Sample Size

Eighteen healthy subjects are planned to be enrolled. Subjects will be randomized to 1 of 4 treatment sequences. Fourteen subjects are required to detect an increase in cumulative NDMA excreted in urine over 24-h following ranitidine administration compared to placebo assuming a 2-fold increase with ranitidine. Using a log-transformed paired comparison of cumulated amounts and 100% coefficient of variability, there is greater than 90% power at a one-sided significance level. Up to 4 subjects may be qualified as replacements, if needed.

4.9.2 Analysis Populations

The PK population will include all subjects who receive study drug and have at least one on-treatment sample. The safety population will include all subjects who receive at least 1 dose of any of the study drugs. The analysis population will include all subjects in the PK population with calculated PK parameters from at least two study periods of the same meal type (i.e., low nitrite/NDMA or high nitrite/NDMA).

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 Pharmacokinetic Analysis

4.9.6.1 Urine Pharmacokinetics

Cumulative amount of NDMA (primary), DMA (exploratory), and ranitidine (exploratory) excreted in urine over 24-h for placebo and ranitidine administration will be calculated for each

subject. All urine over a 24-h period will be collected. Each separate void will be weighed in a tarred contained. The volume of the void will be determined assuming a specific gravity of 1.01. Concentration of each collected sample will be used to determine a cumulative amount excreted as follows:

$Ae_{0\text{-}24} = \sum^{time} Urine_{weight,time} * 1.01 * Conc_{urine,time}$

For both NDMA (primary), DMA (exploratory) and ranitidine (exploratory) measures will be log-transformed and utilize a mixed-effect analysis of variance (ANOVA) approach for comparing geometric means with terms for treatment, period, and sequence as fixed effects. The results will be transformed back to the original scale by exponentiation to provide treatment geometric means. Testing will be one-sided and a significant increase will be concluded if the lower bound of the one-sided 95% interval for the geometric mean ratio excludes 1.

4.9.6.2 Plasma Pharmacokinetics

The PK parameters $AUC_{0\text{-}inf}$, C_{max}, $AUC_{0\text{-}t}$, T_{max}, and K_{el} will be summarized for NDMA, DMA, and ranitidine using descriptive statistics (number of subjects, mean, SD, coefficient of variation [CV], median, minimum, and maximum) for both treatment groups. 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. Calculation of PK parameters will be performed using actual sampling times.

4.9.6.3 Physiologically-Based Pharmacokinetic Modeling

Plasma and urine NDMA, DMA, and ranitidine concentration data may be used to conduct physiologically-based pharmacokinetic modeling to better understand absorption, distribution, metabolism, and elimination of these compounds. These analyses will be described in more detail in a separate analysis plan.

4.9.6.4 Exploratory Urine and Plasma Sample Analyses

Profiling metabolites and other molecular constituents in biofluids may have utility for exploratory research. For example, profiling comparisons of metabolites might inform on what metabolic pathways are influenced. Differential or altered plasma and urinary profiles can be mapped back to specific pathways that may also be more relevant to one or more tissues. Portions of collected blood and urine samples from this study may be used for the generation of such profiles (e.g., metabolomics) as hypothesis-generating analyses. Other exploratory analyses may also be performed. Additional details regarding the statistical methods for the exploratory analyses will be described in a separate protocol.

4.9.7 Meals Assessment

All meals prepared for subjects will utilize foods shown in the literature to have low nitrite/NDMA or high nitrite/NDMA content. Two servings of each low nitrite/NDMA meal and

each high nitrate/NDMA meal will be frozen at -60°C or below to enable potential analysis of nitrite, NDMA and nitrate content. These analyses will be considered hypothesis-generating. Additional details regarding the analytical methods will be described in a separate protocol.

4.9.8 Safety Analyses

4.9.8.1 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.

4.9.8.2 Clinical Laboratory Tests

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.

4.9.8.3 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.

4.9.8.4 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.

4.9.8.5 Physical Examinations

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

4.9.8.6 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.

4.9.9 Interim Analyses

No interim analyses are planned.

4.9.10 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 Statistical Analysis Plan.

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)

FDA staff with primary responsibility for the FDA's involvement with the project (i.e., the FDA Project Lead) will submit the protocol and associated documentation to FDA's Office of the Chief Scientist to facilitate an Institutional Review Board Authorization Agreement (IAA) with FDA as the relying institution. The FDA Project Lead or investigator will provide the local 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 local 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 local 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 local IRB.

Subjects will be informed that they have the right to contact the local 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 local 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(R1) 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 time line 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 ICH E6(R1) (Section 8) 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 45. 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 certain 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.

8. Appendices

8.1. Appendix A – Schedule of Events

Table 8-1: Schedule of Events

NDMA in Ranitidine Medicines Clinical Study - Schedule of Events

	Screening	Check-in / Baseline	Wash-out	Treatment Period 1															Wash-out	Treatment Period 2													
Study Day	-28 to -3	-2	-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	3	3	3	3	3	3	3	3	3	3	3	3	3
Hour				0	0.5	1	1.5	2	3	4	5	6	9	11	12	14	16	24		0	0.5	1	1.5	2	3	4	5	6	9	11	12	14	15	24
Visit	1	2																														
Recruitment/Screening - healthy	X																															
Screening Stipend	X																															
In House Residency		X	X															X	X													X
Meals		X	X	X															X	X												
Informed Consent	X																															
Medical History	X	X																														
Comprehensive Physical exam	X	X																														
Vital Signs	X	X		X															X	X												
Height, Weight		X																														
12-Lead ECG [1]	X	X																														
Clinical Chemistry and Hematology [1]	X	X																														
Urinalysis	X	X																														
SARS-CoV2 Molecular Test		X																														
HIV Test	X																															
Hepatitis Test	X																															
Urine Drug and Alcohol Screen	X	X																														
Pregnancy Test (females only) [2]	X	X																														
FSH (females only)	X	X																														
H. pylon breath test	X																															
Study agent preparation & application - oral				X																X												
Plasma Sample Collection				X	X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X		X		X
Urine Sample Collection [3]				X					X			X	X		X		X	X		X					X			X	X		X	X	X
Concomitant Medications		X		X															X	X												
Adverse Events		X		X															X	X												

1 clinically notable results are repeated

2 serum test

3 Unscheduled urine collections, if any occur, will be collected and processed the same as scheduled urine collections

Schedule of Events – con't

NDMA in Ranitidine Medicines Clinical Study - Schedule of Events

	Wash-out	Treatment Period 3															Wash-out	Treatment Period 4														End of Study
Study Day	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8
Hour		0	0.5	1	1.5	2	3	4	5	6	9	11	12	14	15	24		0	0.5	1	1.5	2	3	4	5	6	9	11	12	14	15	24	
Visit																																
Recruitment/Screening - healthy																																
Screening Stipend																																
In House Residency	X															X	X														X	
Meals	X	X															X	X														
Informed Consent																																
Medical History																																
Comprehensive Physical exam																																X
Vital Signs	X	X															X	X														X
Height, Weight																																X
12-Lead ECG [1]																																X
Clinical Chemistry and Hematology [1]																																X
Urinalysis																																X
HIV Test																																
Hepatitis Test																																
Urine Drug and Alcohol Screen																																X
Pregnancy Test (females only) [2]																																X
FSH (females only)																																
H. pylori breath test																																
Study agent preparation & application - oral		X																X														
Plasma Sample Collection		X	X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X		X		X
Urine Sample Collection [3]		X					X			X	X		X		X	X		X					X			X	X		X		X	X	
Concomitant Medications	X	X															X	X														X
Adverse Events	X	X															X	X														X

1 clinically notable results are repeated

2 serum test

3 Unscheduled urine collections, if any occur, will be collected and processed the same as scheduled urine collections

9. Reference List

FDA Updates and Press Announcements on NDMA in Zantac (ranitidine). https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine

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Zeng T and Mitch WA. 'Oral intake of ranitidine increases urinary excretion of N-nitrosodimethylamine' Carcinogenesis 2016 Jun; 37(6): 625-34