CLINICAL STUDY PROTOCOL

A Double-Blind, Placebo-Controlled, Crossover Study of Sacrosidase for the Treatment of Subjects with Fructan Intolerance

Protocol Number:	SSDXA-14
IND Number:	164227
Compound/Investigational Product:	Sacrosidase Oral Solution 17,000 IU/2 mL or Placebo
Phase:	Feasibility/pilot study
Sponsor:	QOL Medical, LLC 3405 Ocean Drive Vero Beach, FL 32963
Principal Investigator:	John Leung, MD Boston Food Allergy Center 65 Harrison Avenue Suite 201 Boston, MA 02111
Protocol Date:	24 January 2023
Protocol Version:	1.0
Replaces:	N/A

SIGNATURE ON BEHALF OF SPONSOR

Study Title: A Double-Blind, Placebo-Controlled, Crossover Study of Sacrosidase for the Treatment of Subjects with Fructan Intolerance

Protocol Number: SSDXA-14

I have read the protocol SSDXA-14 titled "A Double-Blind, Placebo-Controlled, Crossover Study of Sacrosidase for the Treatment of Subjects with Fructan Intolerance" and confirm that, to the best of my knowledge, the protocol accurately describes the design and conduct of the study.

_____ _____

Company/Sponsor Signatory Date

Weng Tao, MD
QOL Medical, LLC
3405 Ocean Drive
Vero Beach, FL 32963

SIGNATURE OF INVESTIGATOR

Study Title: A Double-Blind, Placebo-Controlled, Crossover Study of Sacrosidase for the Treatment of Subjects with Fructan Intolerance

Protocol Number: SSDXA-14

I have read the protocol SSDXA-14 titled "A Double-Blind, Placebo-Controlled, Crossover Study of Sacrosidase for the Treatment of Subjects with Fructan Intolerance." By signing this protocol, I agree to conduct the clinical study, after approval by an Institutional Review Board or Independent Ethics Committee (as appropriate), in accordance with the protocol, the principles of the Declaration of Helsinki (with amendments), the standards of Good Clinical Practice (as defined by the International Conference on Harmonization), and applicable regulatory requirements.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____
Printed Name of Investigator

_____
Signature of Investigator

_____
Date

1. TABLE OF CONTENTS

2. LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CSID	Congenital Sucrase-Isomaltase Deficiency
ENT	Ears, nose, and throat
FBT	Fructan Breath Test
FDA	Food and Drug Administration
FOS	Fructo-oligosaccharides
GCP	Good Clinical Practice
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IBS	Irritable Bowel Syndrome
ICH	International Conference on Harmonization
ICH E6	International Conference on Harmonization Guidance for Industry, Good Clinical Practice: Consolidated Guidance
IRB	Investigational Review Board
ITT	Intent-To-Treat
LDT	Lab Developed Test
LSMEANS	Least Square Means
MITT	Modified Intention-To-Treat
NSAID	Nonsteroidal Anti-Inflammatory Drug
PI	Principal Investigator
PPM	Parts Per Million
PP	Per-protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TSH	Thyroid Stimulating Hormone
TSS	Total Symptom Score
US	United States
USDA	United States Department of Agriculture

3. PROTOCOL SUMMARY

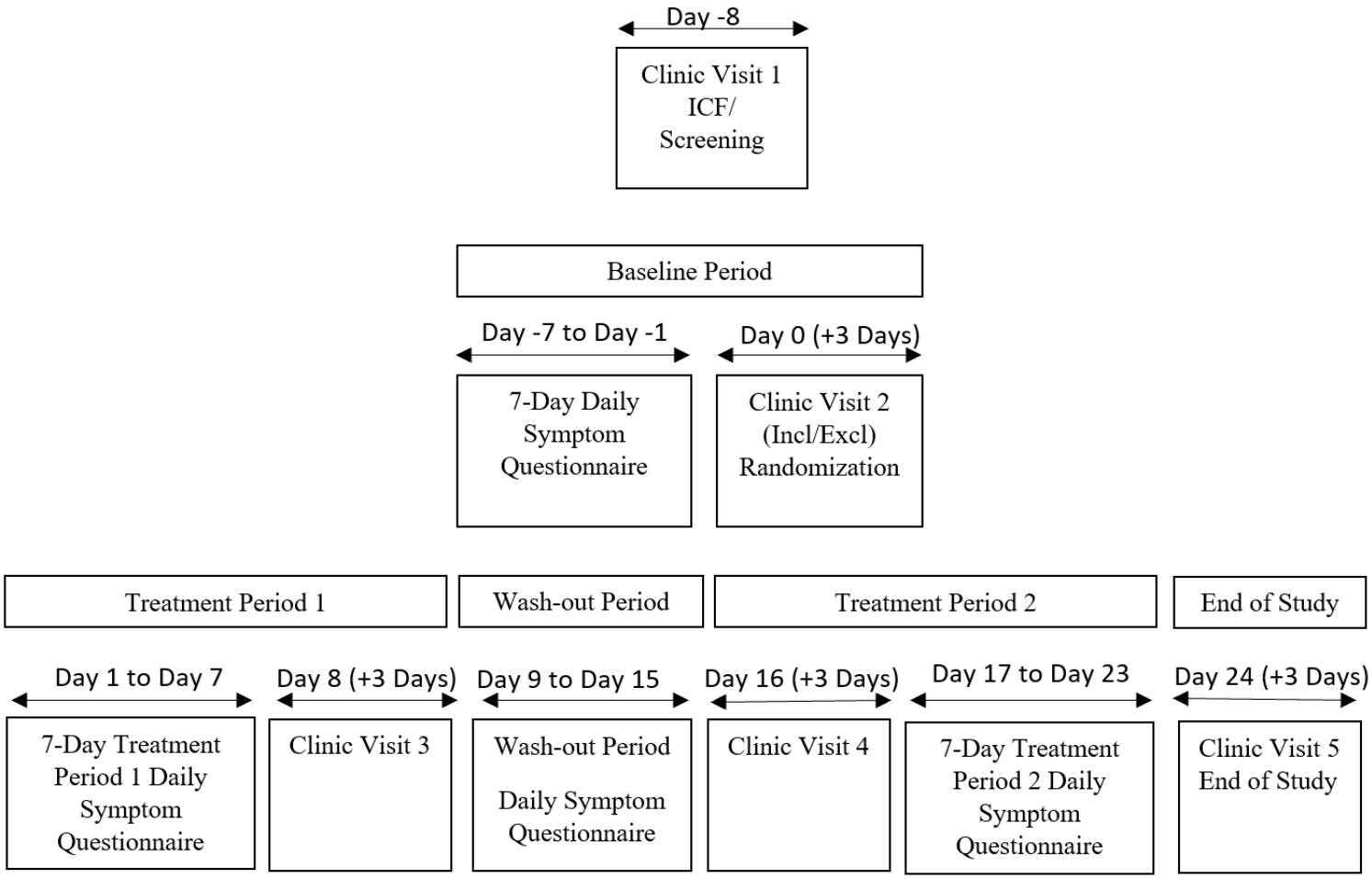
3.1. Synopsis

Title	A Double-Blind, Placebo-Controlled, Crossover Study of Sacrosidase for the Treatment of Subjects with Fructan Intolerance
Number of Sites	1
Study Participation Duration	Up to 7 weeks
Estimated Enrollment	25 participants
Study Objectives	Primary: to determine if sacrosidase is effective in reducing the incidence and severity of gastrointestinal (GI) symptoms in subjects with fructan intolerance Secondary: to determine if sacrosidase is effective in reducing the incidence and severity of abdominal bloating, and to assess the safety and tolerability of sacrosidase compared to placebo in subjects with fructan intolerance
Patient Selection	**Inclusion Criteria** 1. Ability to comprehend and provide a signed and dated informed consent form. 2. Stated willingness to comply with all study procedures and attend all scheduled clinic visits and continue participation for the duration of the study. 3. Ability to self-administer oral medication and willingness to adhere to the medication regimen. 4. Male or non-pregnant, non-lactating female, at least 18 years of age. 5. Sexually active women of childbearing potential must agree to use at least one reliable method of birth control while participating in the study. 6. Presence of fructan intolerance as determined by a positive result on a fructan breath test (FBT) within the last 6 months. 7. Subjects who are lactose intolerant agree to eliminate all lactose from their diet during the study. 8. Stated willingness to discontinue any medications to resolve GI symptoms (digestive enzymes, antacids, proton pump inhibitors, histamine-2 blockers, promotility agents, or anti-diarrheal agents, etc.), per the investigator's discretion during the study. 9. Stated willingness to discontinue any over-the-counter or prescribed oral nonsteroidal anti-inflammatory drugs (NSAIDs) during the study. 10. Per the discretion of the investigator, absence of any GI disorder other than a diagnosis of fructan intolerance. **Exclusion Criteria** 1. History of inflammatory bowel disease or active low-grade GI bacterial infection, as diagnosed by the presence of fecal calprotectin. 2. History of celiac disease, as diagnosed by serology testing for anti-gluten protein antibodies.

	3. History of Congenital Sucrase-Isomaltase Deficiency (CSID), per the discretion of the investigator. 4. Abnormal uncontrolled thyroid function, detected by abnormal thyroid stimulating hormone (TSH) level in the blood. 5. Per the discretion of the investigator, history of a serious physical disorder that prevents the subject from attending study visits and complying with study-related procedures or a mental disorder that prevents the subject's ability to make decisions. 6. BMI greater than 30 kg/m2. 7. History of diabetes. 8. History of hypersensitivity to yeast, yeast products, glycerin (glycerol), or papain. **Randomization Criteria** 1. Subjects must complete the Daily Symptom Questionnaire at least 5 of 7 days during the 7-day Baseline Period. 2. Subjects must have a baseline Total Symptom Score (TSS) of ≥ 3 for men and ≥ 5 for women as determined by the Daily Symptom Questionnaires during the 7-day Baseline Period.
Study Design Description	This is a feasibility/pilot double-blind, placebo-controlled, crossover study to evaluate the efficacy and safety of sacrosidase and placebo in 25 subjects objectively diagnosed with fructan intolerance. This study will consist of a Screening Visit, Baseline Period, Treatment Period 1, Washout Period, and Treatment Period 2. Subjects will be required to attend 5 clinic visits during study participation.
Study Treatment	Subjects will be randomized in a 1:1 fashion to either receive sacrosidase or placebo during Treatment Period 1. Following a 7-day Washout Period, subjects will receive their crossover study medication for Treatment Period 2. Subjects will take their assigned study medication for 7 days during each Treatment Period.
Product, Dosage, and Mode of Administration	Subjects will take either sacrosidase or placebo, 2 mL (17,000 IU) with every meal or snack. The recommended dosage for subjects greater than 15 kg in body weight is 2 mL (17,000 IU) or 2 full measuring scoops (each full measuring scoop equals 1 mL); taken orally with each meal or snack diluted with 4 ounces (120 mL) of water or milk. The beverage should be served cold or at room temperature. The beverage should not be warmed or heated before or after addition of sacrosidase or placebo because heating is likely to decrease potency. Sacrosidase or placebo should not be reconstituted or consumed with fruit juice since its acidity may reduce the enzyme activity. It is recommended that approximately half of the dosage be taken at the beginning of the meal or snack and the remainder be taken during the meal or snack. The recommended dosage for this study is as follows:

	• 2 mL (17,000 IU) (two full measuring scoops or 56 drops) per meal or snack for subjects over 15 kg in body weight.

3.2. Schematic



3.3. Schedule of Events

Event	Screening Period	Baseline Period		Treatment 1 Period		Washout Period		Treatment 2 Period	
Site	**Visit 1**	**Home**	**Visit 2**	**Home**	**Visit 3**	**Home**	**Visit 4**	**Home**	**Visit 5**
Day (Range)	Day -8	Day -7 to Day -1	Day 0 (+ 3 Days)	Day 1 to Day 7	Day 8 (+3 Days)	Day 9 to Day 15	Day 16 (+3 Days)	Day 17 to Day 23	Day 24 (+3 Days)
Informed Consent	X								
Demographics	X[a]								
Medical History	X								
Concomitant Medications	X		X		X		X		X
Vital Signs	X[b]		X[b]		X[b]		X[b]		X[b]
Abbreviated Physical Exam	X[c]		X[c]		X[c]		X[c]		X[c]
Urine Pregnancy	X[d]		X[d]				X[d]		
Enrollment; Randomization	X		X[f]						
Questionnaire Dispensation	X		X		X		X		
Questionnaire Completion		X[e]		X[e]		X[e]		X[e]	
Questionnaire Collection			X		X		X		X
Study Drug Dispensation			X				X		
Study Drug Administration				X				X	
Collection Study Medication					X				X
Evaluation of Adverse Events			X		X		X		X

a. Demographics to include age, gender (assigned at birth), race, and ethnicity.
b. Site will collect the subject's systolic and diastolic blood pressure, heart rate, respiratory rate, temperature, height, and body weight. Subject must sit quietly for at least two minutes prior to collecting systolic and diastolic blood pressure, heart rate, and respiratory rate. Height will only need to be collected at Visit 1.
c. An abbreviated physical exam will be performed including ears, nose, and throat (ENT); respiratory system; and cardiovascular system.
d. A urine pregnancy test will be performed on all female subjects of childbearing potential. Sexually active women of childbearing potential must agree to use a reliable method of birth control while participating in this study. Subjects must have a negative urine pregnancy test at Visit 1, Visit 2, and Visit 4.
e. Subjects should be encouraged to complete the daily questionnaires every day, but subjects must complete the daily questionnaire at least 5 of the 7 days during the baseline and treatment periods, as specified above.
f. Subjects must have a baseline TSS of ≥ 3 for men and ≥ 5 for women, as determined by the Baseline Daily Symptoms Questionnaires.

4. INTRODUCTION

4.1. Study Rationale

The human digestive system lacks enzymes that can break the fructose-fructose glycosidic bonds found in fructo-oligosaccharides (FOS) (Stephanopoulos et al., 1998). Thus, a diet high in fructans may lead to symptoms of carbohydrate malabsorption and other irritable bowel syndrome (IBS)- like symptoms due to bacterial fermentation and increased osmotic load in the large intestine (Shepard et al., 2008; Fedewa & Rao, 2014). Currently, the only way to manage fructan intolerance is by dietary restriction of fructan-containing foods (Fedewa & Rao, 2014).

The exact prevalence of fructan intolerance in the general United States (U.S.) population is unknown, but among patients diagnosed with IBS, 24% are estimated to be sensitive to fructans (Fedewa & Rao, 2014). GI symptoms such as flatulence following consumption of low levels of fructans may be considered within the normal range among the general population, but patients with IBS may experience exaggerated symptoms of painful bloating and flatulence due to a visceral hypersensitivity. (Haller & Scarlata, 2021; Chumpitazi, et al., 2018; Ong, et al., 2010).

There is no validated diagnostic test for fructan intolerance. A 3-hour hydrogen-methane FBT following the administration of fructan 7.5 g to 25 g is currently considered the best objective measure of fructan metabolism. This is an in-house Lab Developed Test (LDT) to detect fructan intolerance. A test finding of an increase from baseline of ≥ 20 ppm of exhaled hydrogen or ≥ 10 ppm of exhaled methane, combined with characteristic GI symptoms experienced during the test, are considered confirmation of the diagnosis of fructan intolerance (Fedewa & Rao, 2014; Ong, et al., 2010).

The objective of this clinical trial is to determine if sacrosidase can relieve GI symptoms of fructan intolerance in subjects who have been objectively diagnosed with fructan intolerance. The underlying hypothesis for this study is that sacrosidase facilitates the metabolism of fructans by enzymatically breaking hydrolytic bonds at the fructose-fructose links found in fructans.

The study drug sacrosidase, at a dose of 2 mL, will be self-administered with every meal or snack. As there is no pharmaceutical standard of care for fructan intolerance, the study will employ a placebo control. In order to control for variables that may be introduced by individual diets and metabolism, the study will be conducted as a crossover study, with each subject serving as the control for themselves.

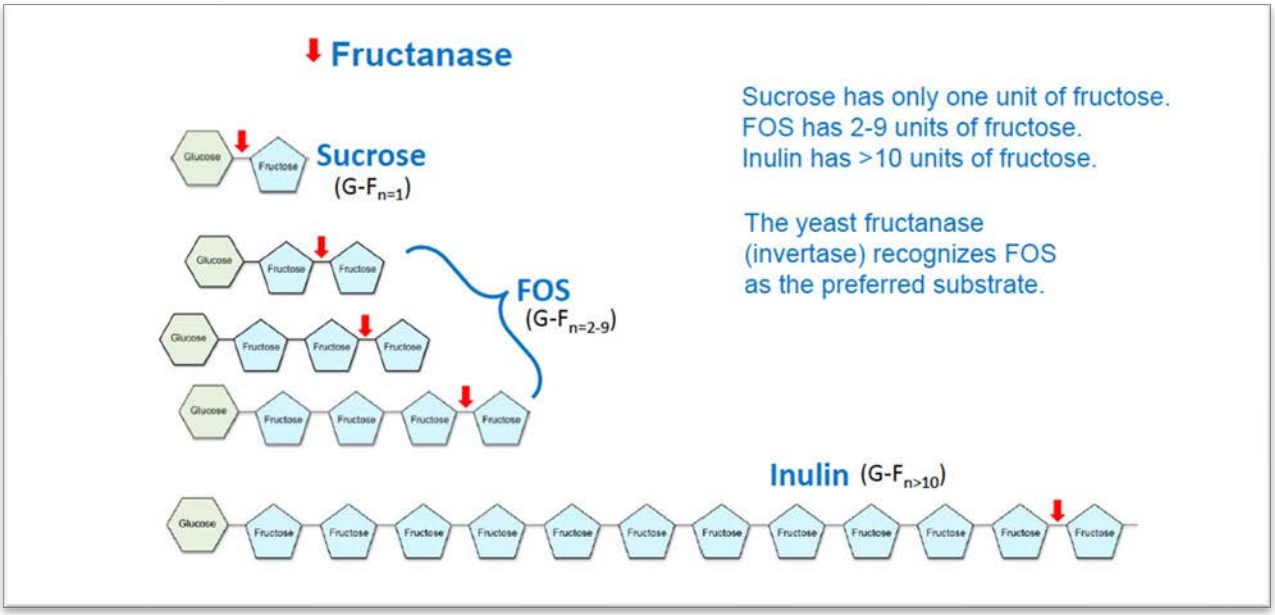
4.2. Background Information

Sacrosidase, an enzyme derived from baker's yeast (*Saccharomyces cerevisiae*), also known as ß,D-fructofuranoside fructohydrolase, is a glycosylated monomeric enzyme consisting of 513 amino acids, with a total molecular weight of 100,000 Da.

The substrates for sacrosidase are carbohydrates comprising a single glucose (G) molecule linked to 1 or more fructose (F) molecule(s), represented as $G\text{-}F_n$, with $n \geq 1$. The simplest carbohydrate is the combination of a single glucose and fructose molecule ($G\text{-}F_1$), commonly known as sucrose. A more complex carbohydrate consists of $G\text{-}F_n$, where $n > 1$ are fructose molecules referred to as FOS. FOS

compounds that contain 2 to 9 units of fructose are called fructans, and FOS compounds that contain 10 or more fructose are called inulin (Hendry & Wallace, 1993; Stick & Williams, 2009; Fedewa & Rao, 2014).

Sacrosidase hydrolyzes the α-1,β-2-glycosidic bond that joins fructose and glucose molecules (F-G), as well as adjacent fructose molecules, as shown in the figure below:



Fructans are highly fermentable, poorly absorbed, short-chain carbohydrates commonly referred to as FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) (Shepard, et al., 2008). Fructans are found in approximately 15% of the flowering plant species, which include wheat, rye, barley, and chicory, and bulb-forming plants such as onions, garlic, leeks, scallions, chives, and shallots (Vijn, et al., 2000). Fructans are also found in fruits (watermelon, grapefruit, nectarine, persimmon, plums, pomegranate, ripe bananas, dates, prunes, and raisins), vegetables (onions, shallots, leeks, asparagus, artichoke, beets, brussels sprouts, savoy cabbage, fennel, and snow peas), legumes (kidney beans, black beans, lima beans, mung beans, navy beans, and split peas), nuts (cashews and pistachios), garlic and inulin (aka chicory root), and some soy products.

The most recent U.S. Department of Agriculture report on the average consumption of fructans by the U.S. population was 3.91 g fructans each day (Fedewa & Rao, 2014). Fructans in the U.S. diet are mostly consumed in the form of wheat (70% of all fructans) and 25% from onions (Moshfegh, et al., 1999).

4.3. Potential Risks and Benefits

4.3.1. Known Potential Risks

Patients with a hypersensitivity to yeast, yeast products, papain, or glycerin (glycerol) may develop a serious allergic reaction to sacrosidase. This may manifest as difficulty breathing,

wheezing, or swelling of the face. Patients who experience a serious allergic reaction to sacrosidase should stop taking sacrosidase and immediately seek emergency medical attention.

Some patients treated with sacrosidase may experience an increase in abdominal pain, vomiting, nausea, or diarrhea. Constipation, difficulty sleeping, headache, nervousness, and dehydration have also occurred in patients treated with sacrosidase.

4.3.2. Known Potential Benefits

It is anticipated that fructan intolerant patients would benefit from sacrosidase treatment and experience reduced GI symptoms.

5. STUDY OBJECTIVE AND ENDPOINTS

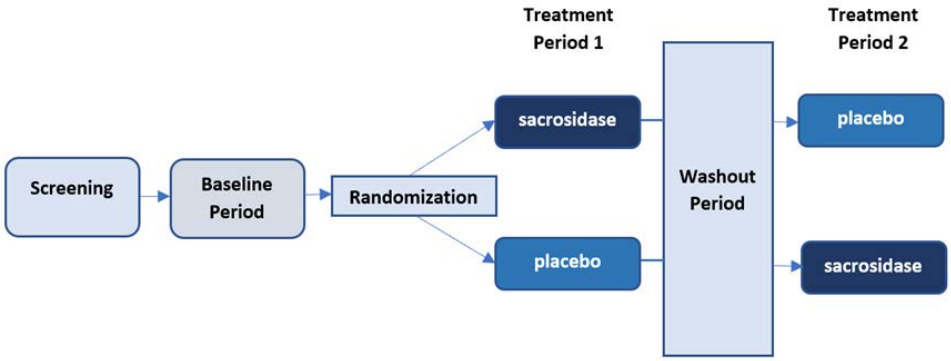
Primary Objective	Primary Endpoint
To determine if sacrosidase is effective in reducing the incidence and severity of GI symptoms in subjects with fructan intolerance.	Primary efficacy endpoint is a change from baseline in the TSS, based on a mean value of combination scores for: • incidence of seven GI symptoms experienced in the past 24 hours during a 7-day period, and • severity of seven GI symptoms experienced in the past 24 hours during a 7-day period.
Secondary Objectives	**Secondary Endpoints**
To determine if sacrosidase is effective in reducing the incidence and severity of abdominal bloating, as determined by the Daily Symptom Questionnaire.	Secondary efficacy endpoint is a change from baseline on incidence and severity of abdominal bloating experienced in the past 24 hours during a 7-day period.
To assess the safety and tolerability of sacrosidase compared to placebo in subjects with fructan intolerance.	The safety endpoint will be assessed by the frequency and severity of treatment emergent adverse events (TEAEs) and Serious Adverse Events (SAEs) and any premature study withdrawals related to adverse reactions.

6. STUDY DESIGN

6.1. Description of the Study Design

This is a feasibility/pilot double-blind, placebo-controlled, crossover study to evaluate the efficacy and safety of sacrosidase and placebo in 25 subjects with fructan intolerance. This study will consist of a Screening Visit, Baseline Period, Treatment Period 1, Washout Period, and Treatment Period 2. The study design is depicted graphically in Figure 1.

Figure 1: Crossover Study Design



6.2. Study Procedures

A bulleted summary of the procedures and assessments at each visit is provided in this section.

6.2.1. Visit 1: Screening Visit (Day -8) - Clinic

Study staff will:

- Obtain informed consent from subjects who had been previously screened in the principal investigator's (PI) clinic for the presence of fructan intolerance and the absence of:
    1) inflammatory bowel disease or low-grade GI bacterial infection, detected by fecal calprotectin test;
    2) celiac disease, detected by serology for anti-gluten protein antibodies;
    3) CSID, per the discretion of the investigator; and
    4) abnormal uncontrolled thyroid function, detected by the standard TSH blood test.
- Collect and record subject demographics to include age, gender (assigned at birth), race, and ethnicity.
- Collect and record subject's vital signs to include systolic and diastolic blood pressure, heart rate, respiratory rate, temperature, height, and body weight.
- Perform an abbreviated physical exam including ears, nose, and throat (ENT); respiratory system; and cardiovascular system.
- Perform a urine pregnancy test for all female subjects of childbearing potential.
- Screen for inclusion/exclusion criteria to determine eligibility for study enrollment.
- Review the instructions and expectations for completing the Daily Symptom Questionnaire for the 7-day Baseline Period.
- Distribute Daily Symptom Questionnaires either electronically or on paper for the 7-day Baseline Period.
- Schedule next study visit, Visit 2.

6.2.2. Baseline Period (Day -7 to Day -1) - Home

Starting the day after Visit 1, subjects will complete the Daily Symptom Questionnaire at the end of each day for 7 consecutive days to establish their baseline TSS values. The Daily Symptom Questionnaire should only be completed from Day -7 to Day -1. A minimum of 5 days must be completed to be eligible for randomization.

6.2.2.1. Visit 2: Baseline Visit (Day 0) – Clinic

Subjects will have a 3-day window from the last day of the Baseline Period to return to the study clinic. During this visit, study staff will:

- Collect the completed Daily Symptom Questionnaires.
- Determine and confirm eligibility for study randomization.
    - Subjects must complete the Daily Symptom Questionnaire at least five days during the Baseline Period and have a baseline TSS of ≥ 3 for men and ≥ 5 for women.
        - Subjects who do not meet the criteria above will be considered a screen fail.
- Document any reported adverse events or changes to medical history.
- Document any changes in concomitant medications.
- Collect and record subject's vital signs to include systolic and diastolic blood pressure, heart rate, respiratory rate, temperature, and body weight.
- Perform an abbreviated physical exam including ears, nose, and throat (ENT); respiratory system; and cardiovascular system.
- Perform a urine pregnancy test for all female subjects of childbearing potential.
- Randomize eligible subject.
- Review the instructions and expectations for completing the Daily Symptom Questionnaire and Dosing Log during the 7-day Treatment Period 1.
- Distribute Daily Symptom Questionnaires and Dosing Log either electronically or on paper for 7-day Treatment Period 1.
- Review the proper dosing and handling requirements for study medication.
- Dispense assigned study medication.
- Schedule next study visit, Visit 3.

6.2.3. Treatment Period 1 (Day 1 to Day 7) – Home

Starting the day after Visit 2, all subjects will self-administer their assigned study medication as instructed with each meal or snack and complete the Daily Symptom Questionnaire and Dosing Log for 7 consecutive days.

- Subjects must complete the Daily Symptom Questionnaire at least 5 days and take their study medication as directed with each meal or snack for at least 5 days during the 7-day Treatment Period 1.
- Subjects who fail to meet the minimum compliance requirements above may be withdrawn from the study.

6.2.3.1. Visit 3 (Day 8) – Clinic

Subjects will have a 3-day window from the last day of Treatment Period 1 to return to the study clinic. During this visit, study staff will:

- Collect the completed Daily Symptom Questionnaires.
    - Confirm Daily Symptom Questionnaire compliance - Subjects must complete the Daily Symptom Questionnaire at least 5 days of the 7 days during Treatment Period 1.
- Collect completed Dosing Log.
    - Confirm study medication compliance – Subjects must take their study medication as directed with each meal or snack for at least 5 days during the 7-day Treatment Period 1.
- Collect unused/used study medication.
- Document any reported adverse events or changes to medical history.
- Document any changes in concomitant medications.
- Collect and record subject's vital signs to include systolic and diastolic blood pressure, heart rate, respiratory rate, temperature, and body weight.
- Perform an abbreviated physical exam including ears, nose, and throat (ENT); respiratory system; and cardiovascular system.
- Review the instructions and expectations for completing the Daily Symptom Questionnaire during the 7-day Washout Period.
- Distribute Daily Symptom Questionnaires either electronically or on paper for the 7-day Washout Period.
    - No study medication will be dispensed at this visit.
- Schedule next study visit, Visit 4.

6.2.4. Washout Period (Day 9 to Day 15) – Home

Starting the day after Visit 3, subjects will complete the Daily Symptom Questionnaire at the end of each day for 7 consecutive days to demonstrate the lack of any treatment carryover effect.

6.2.4.1. Visit 4 (Day 16) – Clinic

Subjects will have a 3-day window from the last day of the Washout Period to return to the study clinic. During this visit, study staff will:

- Collect the completed Daily Symptom Questionnaires.
- Document any reported adverse events or changes to medical history.
- Document any changes in concomitant medications.
- Perform a urine pregnancy test for all female subjects of childbearing potential.
- Collect and record subject's vital signs to include systolic and diastolic blood pressure, heart rate, respiratory rate, temperature, and body weight.
- Perform an abbreviated physical exam including ears, nose, and throat (ENT); respiratory system; and cardiovascular system.
- Review the instructions and expectations for completing the Daily Symptom Questionnaire and Dosing Log during the 7-day Treatment Period 2.

- Distribute Daily Symptom Questionnaires and Dosing Log either electronically or on paper for 7-day Treatment Period 2.
- Review the proper dosing and handling requirements for study medication.
- Dispense assigned study medication.
- Schedule next study visit, Visit 5.

6.2.5. Treatment Period 2 (Day 17 to Day 23) – Home

Starting the day after Visit 4, all subjects will self-administer their assigned study medication as instructed with each meal or snack and complete the Daily Symptom Questionnaire and Dosing Log for 7 consecutive days.

- Subjects must complete the Daily Symptom Questionnaire at least 5 days and take their study medication as directed with each meal or snack for at least 5 days during the 7-day Treatment Period 2.
- Subjects who fail to meet the minimum compliance requirements above may be withdrawn from the study.

6.2.5.1. Visit 5: End of Study (Day 24) – Clinic

Subjects will have a 3-day window from the last day of Treatment Period 2 to return to the study clinic. During this visit, study staff will:

- Collect the completed Daily Symptom Questionnaires.
    - Confirm Daily Symptom Questionnaire compliance - Subjects must complete the Daily Symptom Questionnaire at least 5 days of the 7 days during Treatment Period 2.
- Collect completed Dosing Log.
    - Confirm study medication compliance - Subjects must take their study medication as directed with each meal or snack for at least 5 days during the 7-day Treatment Period 2.
- Collect unused/used study medication.
- Collect and document any reported adverse events or changes to medical history.
- Collect and document any changes in concomitant medications.
- Collect and record subject's vital signs to include systolic and diastolic blood pressure, heart rate, respiratory rate, temperature, and body weight.
- Perform an abbreviated physical exam including ears, nose, and throat (ENT); respiratory system; and cardiovascular system.

6.2.6. Unscheduled Visits

Subjects who return to the clinic between scheduled study visits will have an unscheduled visit performed. The primary reason and date for the visit must be documented. No specific tests or procedures are required at this visit, but any tests or procedures that are done at this visit per the investigator's judgment will be documented.

7. STUDY POPULATION

The pool of potential study subjects will be selected from the PI's clinic population and will have met several prior inclusion criteria in order to be considered for study participation. These prior screens were for:

- Presence of fructan intolerance;
- Normal thyroid function, based on a normal TSH level obtained within one year prior to study enrollment;
- Absence of inflammatory bowel disease;
- Absence of GI bacterial infection;
- Absence of celiac disease; and
- Absence of CSID.

The presence of fructan intolerance will be based on a hydrogen-methane FBT. A 3-hour hydrogen-methane FBT following the administration of fructan (25 g) is currently considered the best objective measure of fructan metabolism. A test finding of an increase from baseline of ≥ 20 ppm of exhaled hydrogen or ≥ 10 ppm of exhaled methane, combined with characteristic GI symptoms experienced during the test, are considered confirmation of the diagnosis of fructan intolerance (Fedewa & Rao, 2014). This is an in-house LDT to detect fructan intolerance.

The test for inflammatory bowel disease or low-grade GI bacterial infection will be the fecal calprotectin test; the test for celiac disease will be serology for anti-gluten protein antibodies; and the test for CSID will be to the discretion of the investigator and may include the sucrose hydrogen-methane breath test, ^{13}C-sucrose breath test, sucrose challenge test, genetic test, or disaccharidase assay. The test for thyroid function, detected by the TSH level, will be measured within one year before the study enrollment, by the standard blood test.

Women and members of minority groups and their subpopulations will be free to participate in the screening for this study, in accordance with the NIH Policy on Inclusion of Women and Minorities as Participants In Research Involving Human Subjects.

7.1. Inclusion Criteria

The potential study subjects who give their informed consent to participate in this clinical study must meet all of the following inclusion criteria in order to be enrolled in the study:

1. Ability to comprehend and provide a signed and dated informed consent form.
2. Stated willingness to comply with all study procedures, attend all scheduled clinic visits, and continue participation for the duration of the study.
3. Ability to self-administer oral medication and willingness to adhere to the medication regimen.
4. Male or non-pregnant, non-lactating female, at least 18 years of age.
5. Sexually active women of childbearing potential must agree to use at least one reliable method of birth control while participating in the study.
6. Presence of fructan intolerance as determined by a positive result on a FBT within the last 6 months.

7. Subjects who are lactose intolerant agree to eliminate all lactose from their diet during the study.
8. Stated willingness to discontinue any medications to resolve GI symptoms (digestive enzymes, antacids, proton pump inhibitors, histamine-2 blockers, promotility agents, or anti-diarrheal agents, etc.), per the investigator's discretion.
9. Stated willingness to discontinue any over-the-counter or prescribed NSAIDs during the study.
10. Per the discretion of the investigator, absence of any GI disorder other than a diagnosis of fructan intolerance.

7.2. Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. History of inflammatory bowel disease or active low-grade GI bacterial infection, as diagnosed by the presence of fecal calprotectin;
2. History of celiac disease, as diagnosed by serology testing for anti-gluten protein antibodies;
3. History of CSID per the discretion of the investigator;
4. Abnormal uncontrolled thyroid function, detected by abnormal TSH level in the blood;
5. Per the discretion of the investigator, history of a serious physical disorder that prevents the subject from attending study visits and complying with study-related procedures or a mental disorder that prevents the subject's ability to make decisions;
6. BMI greater than 30 kg/m2;
7. History of diabetes; and/or
8. History of hypersensitivity to yeast, yeast products, glycerin (glycerol), or papain.

7.3. Randomization Criteria

Study subjects must meet the following criteria in order to be randomized into the study:

1. Complete the Daily Symptom Questionnaire at least 5 of 7 days during the 7-day Baseline Period.
2. Subjects must have a baseline TSS of ≥ 3 for men and ≥ 5 for women as determined by the Daily Symptom Questionnaires during the 7-day Baseline Period.

7.4. Prohibited Medications

Subjects will be asked to refrain from using any medications to resolve GI symptoms such as digestive enzymes, antacids, proton pump inhibitors, histamine-2 blockers, promotility agents, or anti-diarrheal agents. Subjects will also be required to refrain from the use of any over-the-counter or prescribed oral NSAIDs during the study.

7.5. Permitted Medications

Any subjects who have been prescribed an antidepressant, such as one of the tricyclic antidepressants, to manage their IBS symptoms may continue with their therapy as long as there has been no change in dosage within the last 30 days prior to informed consent. Any other prescription or over-the-counter medications will be considered per the investigator's judgement.

7.6. Dietary Restrictions

Study subjects will be free to continue with their normal diet during the study, except those who are lactose intolerant, who must agree to eliminate all lactose from their diet during the study.

7.7. Screen Failures

Any subject who signs the informed consent form and fails to meet all of the inclusion criteria or meets any of the exclusion criteria at study Visit 1 will be considered a screen fail. Subjects who fail to complete the Daily Symptom Questionnaire at least 5 of the 7-day Baseline Period and/or does not have a baseline TSS of ≥ 3 for men and ≥ 5 for women as determined by the Daily Symptom Questionnaires during the 7-day Baseline Period will not be eligible for randomization and will be considered a screen fail.

Any subject who does not meet enrollment criteria after signing the informed consent form may be rescreened once per the investigator's discretion.

8. DISCONTINUATION

8.1. Subject Discontinuation/Withdrawal from the Study

A non-completing subject is defined as one who exits the study voluntarily or at the discretion of the investigator and/or the sponsor prior to completing all study visits. Any subject may decide to voluntarily withdraw from the study at any time without prejudice and the reason will be documented accordingly. In the event that discontinuation of study medication is necessary, the investigator will make every attempt to complete all subsequent safety assessments as outlined in Visit 5 (End of Study Visit). Every attempt should be made to keep subjects in the study and to perform the required study procedures, but if this is not possible, the subject may be withdrawn. Subjects may be withdrawn from the study for any reason at any time, including but not limited to the following reasons:

- Withdrawal of consent;
- Any clinical adverse event, including pregnancy and COVID;
- Investigator judgement;
- Lack of efficacy;
- Lack of compliance with study medication and/or procedures; and/or
- Subject is lost to follow-up.

The reason for subject discontinuation or withdrawal from the study will be documented accordingly.

In the event of study discontinuation (other than lost to follow-up), the investigator should make every attempt to have the subject complete Visit 5 (End of Study Visit). The reason for premature discontinuation should be recorded in the subject chart and documented accordingly. If additional therapy is prescribed by the investigator, it must also be recorded in the subject chart and entered in the concomitant medication log.

8.2. Subject Discontinuation Related to Adverse Events or Serious Adverse Events

Subjects may be withdrawn from the study at any time if they experience adverse events or serious adverse events. Severe allergic reaction can happen in people taking sacrosidase. Symptoms of a severe allergic reaction include difficulty breathing, wheezing, swelling of the face, lips, mouth, or tongue. Subjects should discontinue their study medication and contact their study site immediately should they experience a severe allergic reaction. Subjects who experience a severe allergic reaction will be withdrawn from the study. Other side effects of sacrosidase may include stomach (abdominal pain), vomiting, nausea, diarrhea, constipation, difficulty sleeping, headache, nervousness, or dehydration. These are not all of the possible side effects of sacrosidase. Subjects should report if they are experiencing any suspected or unsuspected adverse events so the investigator can determine the best course of action.

8.3. Discontinuation of Study Medication

The study medication may be discontinued for any subject by the investigator at any time. In the event of discontinuation of study medication, the investigator should make every attempt to have the subject complete a safety follow-up visit 30 days following their last dose of study medication. The subject should come in for an End of Study visit (Visit 5) following their last dose. If a subject discontinues study medication prematurely, the reason for premature discontinuation should be recorded in the subject chart and documented appropriately.

8.4. Handling of Subject Withdrawal or Termination

In order to be considered evaluable for efficacy, a subject must have self-administered the study drug during Treatment Period 1 and Treatment Period 2 and have completed Daily Symptom Questionnaires for at least 5 of 7 days per week during the Baseline Period, Treatment Period 1, and Treatment Period 2 in this crossover study.

In order to evaluate for safety, subjects who self-administer at least 1 dose of study medication (sacrosidase or placebo) will be expected to report all occurrences of AEs or SAEs.

The reason for a premature withdrawal will be evaluated by the PI to determine if the withdrawal is possibly or probably related to the study treatment. Tolerability of the study treatment will be determined by the percentage of subjects who complete the study and by the number of withdrawals determined to be possibly or probably related to the study treatment.

8.5. Lost to Follow-up

A subject will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff. Reasonable efforts will be made to contact a subject who fails to attend any follow-up appointments, in order to ensure that he/she is in satisfactory health.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit. The site will counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8.6. Study/Site Termination

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further study intervention development;
- The study will be terminated if 2 subjects are prematurely withdrawn from the study due to experiencing a severe allergic reaction.

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IRB or local health authorities, the sponsor's procedures, or GCP guidelines;
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator; or
- Total number of participants included earlier than expected.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

9. STUDY MEDICATION

The active study medication, sacrosidase, is an FDA-approved medication and will be provided by QOL Medical, LLC, the pharmaceutical company that manufactures sacrosidase and is the sponsor of this study. The vehicle for sacrosidase without the addition of sacrosidase will serve as the placebo control and will also be provided by QOL Medical, LLC.

9.1. Formulation, Appearance, Packaging, and Labeling

Both active sacrosidase and placebo are a pale yellow to colorless, clear solution with a pleasant, sweet taste. Both active and placebo sacrosidase is available in 118-mL (4 fluid ounces) multiple-dose, translucent plastic bottles. Each mL of solution contains 8,500 International Units of sacrosidase. A 1-mL measuring scoop is provided with each bottle.

For the purposes of blinding, both the active study medication and the placebo control will be supplied in identical looking bottles labelled with a unique code or kit number. The randomization code will be kept by an independent biostatistician or designee, who is not involved in the day-to-day conduct of the study. Only after the study has been completed and the database locked will the randomization code be broken.

9.2. Product Storage and Stability

Study medication should be stored under refrigeration at 2°C to 8°C (36°F to 46°F). The bottles should be protected from heat and light. Subjects must return any unused and used medication to their study clinic at Visit 3 and Visit 5.

9.3. Preparation

Both the active study medication sacrosidase and the placebo control should be prepared by the subject in the same manner for each dose. As the subjects in this study are all adults who weigh more than 33 pounds, each treatment dose is 2 mL of the oral solution mixed into a 4-ounce (120 mL) beverage. The beverage should be milk or water that is cold or at room temperature, and never warmed before or after the addition of the dose. The study medication should not be mixed or consumed with fruit juice. Heated or acidic beverages can reduce the enzymatic activity of sacrosidase.

The study medication dose can be achieved by using the provided 1 mL scoop to measure 2 scoopfuls of the solution or by adding 56 drops of the solution directly from the bottle. The combination of the study medication dose and beverage should be mixed well before drinking. The measuring spoon should be rinsed with water after each use.

9.4. Dosing and Administration

Both the active study medication, sacrosidase, and the placebo control should be self-administered in the same manner. The study medication should be self-administered every time the subject consumes a snack or meal. Half of the study medication mixture should be consumed at the beginning of every snack or meal, and the other half during the snack or meal.

9.5. Route of Administration

The route of administration for both the active study medication, sacrosidase, and the placebo control is by mouth.

9.6. Dosing Schedule

The dosing schedule for both the active study medication, sacrosidase, and the placebo control is to self-administer the study medication mixture in conjunction with every snack or meal that is consumed each day for 7 days during Treatment Period 1 and Treatment Period 2.

9.7. Duration of Therapy

The duration for EACH of the two treatment periods is one week or 7 days.

9.8. Tracking of Dose/Compliance

Subjects will document the date, time, and meal type (breakfast, lunch, dinner, or snack) with each dose of study medication during Treatment Period 1 and Treatment Period 2.
All used and unused study medication will be collected during Visit 3 and Visit 5, following each treatment period.

10. STUDY ASSESSMENTS

10.1. Efficacy Assessments

10.1.1. Daily Symptom Questionnaire Background

In a typical Western diet, carbohydrates provide the most calories yet may pose significant challenges in their digestion and absorption.

Maldigestion and malabsorption of carbohydrates play an important role in the bacterial metabolism of incompletely absorbed carbohydrates in the colon. These metabolic products have been incriminated in the pathogenesis of a variety of functional abdominal symptoms, including abdominal pain, bloating, flatulence, and altered bowel habits. Many patients express a belief that their symptoms result from allergy or intolerance to them.

Carbohydrate digestion is comprised of 2 stages: a) luminal, in which large, branched starches are broken down into smaller polysaccharides and monosaccharides; and b) mucosal, in which all products of carbohydrate digestion are reduced by brush-border enzymes to monosaccharides, which then are absorbed across the intestinal epithelium via active transport mechanisms.

The primary effectors of mucosal digestion are sucrase-isomaltase, lactase-phlorizin hydrolase, and maltase-glucoamylase, which lead to the generation of glucose, galactose, and fructose. The problem is that there are limits to the capacities of these processes as illustrated by the worldwide prevalence of lactose intolerance related to an age-related, but ethnically variable, decrease in lactase activity from weaning onward.

Recently, there has been much focus on fermentable oligosaccharides, monosaccharides, and polyols, a group of compounds that includes fructans, lactose, fructose, and sorbitol, and that are poorly absorbed yet readily fermentable.

Their fermentation by anaerobic bacteria in the colon ultimately leads to the production of lactic acid and the short-chain fatty acids (SCFAs) acetate, propionate, and butyrate (with an accompanying lowering of colonic luminal pH) and the gases hydrogen, methane, and carbon dioxide. SCFAs exert a variety of effects on motility and intestinal secretion, which can lead to the development of abdominal cramps and diarrhea.

The common clinical symptoms of carbohydrate maldigestion and malabsorption include abdominal pain, cramping, flatulence, bloating, and alteration in bowel habits. These symptoms result: 1) from the increased osmotic load generated, in the gut lumen, by unabsorbed carbohydrates; and 2) from their metabolism by colonic bacteria with the production of hydrogen, methane, and carbon dioxide, which can result in bloating, distension, and flatulence, as well as to SCFAs, which may precipitate cramps and diarrhea.

QOL Medical, LLC sponsored and recently completed (2022) a sucrose challenge symptoms test that was self-administered in adults previously diagnosed with CSID (n=44) and in asymptomatic controls (n=126). Participants consumed 50 g of dissolved sucrose and assessed the severity of abdominal pain, abdominal bloating, nausea, borborygmi, flatulence, and diarrhea every 30 minutes for 4 hours using a 10-point Likert-based questionnaire. The most severe symptom changes were observed at 1 and 2 hours. The sum in the change-from-baseline Likert scores for all 6 symptoms at 1 and 2 hours combined was significantly larger in CSID patients ($p<0.0001$). Upon review of the ROC curves, the biostatistics team noted a gender distinction in terms of the optimum cutoff for maximizing sensitivity and specificity (Table 1).

10.1.2. Table 1: Summary of ROC analysis for Sucrose Challenge Test by Gender

	Male N=39	**Female** N=135
SCT – sum of symptom severity delta – 120 min		
AUC	0.93	0.83
Std Err	0.041	0.040
95% CI - upper	1.000	0.905
95% CI - lower	0.850	0.746
Youden Index	0.83	0.64
Suggested cutoff	3.00	5.00
Sensitivity	1.00	0.84
Specificity	0.83	0.80

Although fructan is a different carbohydrate from sucrose, they share the same pathogenesis for intolerance. The fructans challenge test uses a similar mechanism to assess the same symptoms.

10.1.3. Daily Symptom Questionnaire

All subjects will be required to complete a Daily Symptom Questionnaire either electronically or on paper on a daily basis for 7 consecutive days during the Baseline Period, Treatment Period 1, Washout Period, and Treatment Period 2. The objective of the study is to determine if

sacrosidase is more effective than a placebo in alleviating seven GI symptoms in subjects with fructan intolerance. The primary outcome measure is the TSS. The TSS is a number calculated based on the subject's responses to the Daily Symptom Questionnaire that is to be completed during four time periods in the study: Baseline, Treatment 1, Washout, and Treatment 2.

The results from the Daily Symptom Questionnaire for the frequency and severity of GI symptoms will be used to calculate a single composite TSS for each 7-day assessment period. The TSS calculation is the mean of the summation of the daily symptom scores recorded by the subjects. Each subject who completes the study will have four separate TSS values, the Baseline Period, Treatment Period 1, Washout Period, and Treatment Period 2.

The first part of the Daily Symptom Questionnaire measures the frequency of each of seven GI symptoms experienced, recorded at the end of each day over a 7-day assessment period, on a 6-point Likert scale. The seven GI symptoms are abdominal bloating, abdominal pain, abdominal cramps, abdominal gas/flatulence, diarrhea, constipation, and nausea.

The second part of the Daily Symptom Questionnaire measures the severity of each of these seven GI symptoms experienced, recorded at the end of each day over the same 7-day assessment period, on a 10-point Likert scale.

By comparing the mean change from baseline TSS to the TSS during treatment with the active study medication, sacrosidase, with the same value during treatment with the placebo control, the investigators will be able to test the study hypothesis: treatment with sacrosidase reduces the GI symptoms of fructan intolerance.

10.1.3.1. Daily Symptom Questionnaire Compliance

Subjects should be encouraged to complete their Daily Symptom Questionnaire at the end of every day during each 7-day study period, Baseline Period, Treatment Period 1, and Treatment Period 2. In order to be considered evaluable for efficacy, a subject must have completed the Daily Symptom Questionnaires for at least 5 of 7 days during the Baseline Period and during both Treatment Period 1 and Treatment Period 2.

10.1.4. Dosing Log

All subjects who meet criteria for study randomization will be required to complete a Dosing Log either electronically or on paper on a daily basis for 7 consecutive days during Treatment Period 1 and Treatment Period 2. Subjects will log the date, time, and meal type for each dose of study medication during Treatment Period 1 and Treatment Period 2.

10.1.4.1. Study Medication Compliance

Subjects should be encouraged to take their assigned study medication as directed with each meal or snack for 7 consecutive days during Treatment Period 1 and Treatment Period 2. In order to be considered evaluable for efficacy, a subject must have self-administered

the study medication with each meal or snack for at least 5 of 7 days during both Treatment Period 1 and Treatment Period 2.

10.2. Other Assessments

10.2.1. Demographics

Demographics data will be collected including height, weight, sex, date of birth, race, and ethnic origin (to the extent allowed by local regulations).

10.2.2. Medical History

Information on clinically significant previous and concomitant illnesses, any clinically significant signs or symptoms that are present before informed consent, any pre-existing conditions identified through findings from assessment(s), and self-reported information done during the Screening Visit will be recorded as medical history at screening.

10.2.3. Prior and Concomitant Medications

Concomitant medications are prescription and over-the-counter drugs and supplements a study participant has taken during study participation. This information may be collected as a history item as well as during the study. This includes prior medications that were taken by the subject within 30 days of study enrollment. All prior and concomitant medications taken during the study must be documented. The information must include trade or international non-proprietary name of medication, indication, daily dose, route of administration, and start and end date of administration (if applicable).

11. SAFETY ASSESSMENTS

11.1. Vital Signs

Vital signs, including systolic and diastolic blood pressure, heart rate, respiratory rate, temperature (oral or temporal), height, and body weight, will be collected at Clinic Visits 1, 2, 3, 4, and 5. Prior to collection of systolic and diastolic blood pressure, heart rate, and respiratory rate, the subject should sit quietly for approximately 2 minutes. Blood pressure should be taken in a seated or recumbent position. Height and weight will be measured without shoes. Height will only need to be collected at Clinic Visit 1.

11.2. Abbreviated Physical Exam

An abbreviated physical exam including ears, nose, and throat (ENT); respiratory system; and cardiovascular system will be performed at Clinic Visits 1, 2, 3, 4, and 5.

11.3. Pregnancy Testing

All female subjects of childbearing potential will have a urine pregnancy test performed at Visit 1 prior to study enrollment. All female subjects of childbearing potential who are eligible for randomization will have a urine pregnancy test performed at Visit 2 and Visit 4 prior to dispensing any study medication.

11.4. Adverse Events

Safety and tolerability will be assessed by the frequency and severity of TEAEs, SAEs, and any premature study withdrawals related to adverse reactions. Adverse events will be monitored by the investigators during scheduled clinic visits, unscheduled clinic visits, and by phone as needed throughout the duration of the study.

11.4.1. Definition of Adverse Events (AE)

An AE is any untoward medical occurrence in a subject or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Note: An AE can therefore be any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research will be documented.

Events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition, including either an increase in frequency and/or intensity of the condition;
- New conditions detected or diagnosed after study drug administration even though it may have been present prior to the start of the study;
- Signs, symptoms, or clinical sequelae of a suspected interaction; or
- Signs, symptoms, or clinical sequelae of a suspected overdose of either study drug or a concomitant medication (overdose per se will not be reported as an AE/SAE).

Events that do not meet the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE;
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital);
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not significantly worsen; or
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless rescue medication or other medical treatment is required.

11.4.2. Classification of an Adverse Event

11.4.2.1. Severity of Adverse Event

The severity of all AEs will be assessed by the study investigators, using the following AE severity grading system:

- Mild – Events that require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate – Events that result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events that interrupt a subject's usual daily activities and may require systemic drug therapy or other intervention. Severe events are usually potentially life-threatening or incapacitating.

11.4.2.2. Relationship to Study Medication

The relationship of AEs will be assessed by the PI, using the following guidelines:

- Not Related – The AE is completely independent of the study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the investigator.
- Unlikely Related – A clinical event whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).
- Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information.
- Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

11.4.3. Expected/Known Common Adverse Reactions

Based on findings reported during the sacrosidase developmental studies and years on the market, there are a number of adverse reactions that may occur in patients treated with the active study drug, sacrosidase. These potential adverse reactions are listed in Section 4.3.1.

11.5. Definition of Serious Adverse Events (SAEs)

A serious adverse event is any untoward medical occurrence that, at any dose:

- Results in death;
- Is life-threatening;

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death if it were more severe.

- Requires hospitalization or prolongation of existing hospitalization;
  NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in disability/incapacity; or
  NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect.

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

11.6. Method of AE and SAE Identification and Recording

All AEs, SAEs, and their outcomes must be reported to the appropriate regulatory authority and the Institutional Review Board (IRB) as required by the IRB, federal, state, or local regulations, and governing health authorities, and documented appropriately. The study staff will record all AE reports, and the PI will evaluate all AE reports for severity and relationship to the study medication and identify any SAEs. All AEs and SAEs that occur after the subject has signed informed consent and enrolled into the study, regardless of causality or seriousness, will be assessed and recorded.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE, not the individual signs/symptoms.

Each separate AE episode must be recorded. For example, if an AE resolves completely or resolves to baseline and then recurs or worsens again, this must be recorded as a separate AE. For AEs to be considered intermittent, the events must be of similar nature and severity.

11.7. Regulatory Reporting Requirements for SAEs

Prompt notification of SAEs by the investigator to the study sponsor is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

The study sponsor has a legal responsibility to notify the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. The study sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB, and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and the study sponsor policy and are forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from the study sponsor will file it with the Clinical Investigator's Brochure and will notify the IRB, if appropriate according to local requirements.

12. STATISTICAL CONSIDERATIONS

Statistical methods will be further detailed in a statistical analysis plan (SAP) and approved by the sponsor prior to any analysis. Procedures detailed in the SAP will supersede protocol-specified statistical methods in the event of divergence.

12.1. Primary Efficacy Endpoint

The study hypothesis tested by the primary efficacy endpoint is that the GI symptoms associated with fructan intolerance are reduced when sacrosidase is self-administered with every snack or meal.

The primary efficacy endpoint, a change from baseline TSS and the TSS obtained during treatment with sacrosidase versus the TSS obtained during treatment with placebo, will include these units of analysis:

- Statistical significance of the change in TSS from Baseline and sacrosidase during Treatment Period 1;
- Statistical significance of the change in TSS from Baseline and placebo during Treatment Period 1;
- Statistical significance of the change in TSS from Baseline and sacrosidase during Treatment Period 2; and
- Statistical significance of the change in TSS from Baseline and placebo during Treatment Period 2.

A randomization code for allocating the study medication will be prepared by an independent biostatistician or designee, who is not involved in the day-to-day conduct of the study. Subjects will be randomized in a 1:1 ratio to receive sacrosidase or placebo in a cross-over study design.

If the change from baseline TSS with sacrosidase has a statistical significance of $P < 0.05$ or greater, the study results will be considered confirmation of the hypothesis that sacrosidase can improve the GI symptoms of individuals objectively diagnosed with fructan intolerance.

12.2. Secondary Endpoints

The secondary efficacy endpoint, a change from baseline on incidence and severity of abdominal bloating experienced in the past 24 hours during a 7-day period, will include these units of analysis:

- Change in abdominal bloating from Baseline and sacrosidase during Treatment Period 1;
- Change in abdominal bloating from Baseline and placebo during Treatment Period 1;
- Change in abdominal bloating from Baseline and sacrosidase during Treatment Period 2; and
- Change in abdominal bloating from Baseline and placebo during Treatment Period 2.

The safety endpoint will be assessed by the frequency and severity of TEAEs and SAEs, and any premature study withdrawals related to adverse reactions.

12.3. Analysis Populations

Subject inclusion into each population will be determined prior to the final analysis.

12.3.1. Intention-to-Treat (ITT)

The intent-to-treat (ITT) study population will include all enrolled subjects who underwent randomization, which will occur during Clinic Visit 2.

12.3.2. Modified Intention-to-Treat (mITT)

The modified intention-to-treat (mITT) study population will include all randomized subjects who took at least one dose of sacrosidase and provided completed questionnaires for at least one day of that treatment period (Treatment 1 for Group 1 and Treatment 2 for Group 2).

12.3.3. Safety Analysis Dataset

The safety analysis population will include all subjects who took at least one dose of sacrosidase during Treatment 1 for Group 1 and Treatment 2 for Group 2.

12.3.4. Per-Protocol (PP) Analysis Dataset

The per-protocol population will be the subset of the ITT population who complied with the protocol sufficiently to ensure that these data would be likely to represent the effects of treatment according to the underlying scientific rationale. This will be those subjects who used sacrosidase with at least one meal on every day of the treatment period.

12.4. Description of Statistical Methods

12.4.1. General Approach

Paired t-tests will be used to evaluate the difference between sacrosidase and placebo in the mean change from baseline scores. The mean difference will be reported together with two-sided 95% confidence limits.

A 2x2 crossover analysis model that accounts for period and sequence effects will also be applied. Specifically, the primary efficacy endpoint (change from baseline TSS) will be evaluated using a linear mixed effects analysis of variance (ANOVA) model, including treatment (sacrosidase or placebo), treatment periods (1 or 2), and sequence as fixed effects and subjects as a random effect. Analyses of effects during the treatment periods and carryover effects during the Washout Period will be performed to determine the validity of the crossover design. In the final analysis, if the carryover effect is significant, with a statistical significance of $P < 0.05$, inferences regarding efficacy will be based on Treatment 1 data.

12.4.2. Analysis of the Primary Efficacy Endpoint

For each subject, the mean TSS will be calculated for each study period, based on subject-reported daily questionnaires during these periods:

- Baseline Period;
- Treatment Period 1;
- Washout Period; and
- Treatment Period 2.

The unit of analysis for the primary efficacy endpoint will be the change from the TSSestablished during the Baseline Period and the TSS recorded during the Treatment Period. For all analyses, assessments obtained during the Washout Period will serve to measure any carryover effect by comparing outcomes with those obtained during the Baseline Period.

12.4.3. Analysis of the Secondary Endpoints and Subgroup Analyses

Statistical methods will be further detailed in the SAP.

12.5. Sample Size

Assuming approximately 15% of subjects will not be evaluable, a total of 25 subjects will be enrolled to provide 21 evaluable subjects. A total of 21 evaluable subjects will provide approximately 80% power to detect a mean difference of 2.0 between response to sacrosidase and placebo in the primary endpoint (mean change from baseline in the daily TSS), based on a two-sided t-test with a statistical significance of $P < 0.05$, assuming a standard deviation of 3.0 for paired differences.

12.6. Measures to Minimize Bias

12.6.1. Enrollment/Randomization/Masking Procedures

The enrolled subjects will be randomized during Clinic Visit 2 in a 1:1 fashion to either receive sacrosidase or a placebo. Double blinding will be achieved by distributing the active study medication and the placebo control in identical-looking bottles. The active study drug will be sacrosidase and the placebo will be the vehicle for sacrosidase.

The randomized study medication code for sacrosidase/placebo will be kept by an independent biostatistician or designee, who is not involved in the day-to-day conduct of the study. Only after the study has been completed and the data analyzed will the treatment code be broken.

13. ETHICS/PROTECTION OF SUBJECTS

13.1. Ethical Standard Statement

The study will be conducted in accordance with all applicable regulatory requirements and in accordance with GCP, all applicable subject privacy requirements, and the guiding principles of the Declaration of Helsinki, including, but not limited to:

- IRB review and approval of study protocol and any subsequent amendments;
- Subject informed consent; and
- Investigator reporting requirements.

13.2. Institutional Review Board

The protocol, informed consent forms, recruitment materials, and all subject materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form will be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented subjects need to be re-consented.

13.3. Informed Consent Process

Before being admitted to the study, informed consent will be obtained from each subject (or his/her legally authorized representative) according to the regulatory and legal requirements of the U.S. This consent form must be dated and retained by the investigator as part of the study records. Should a protocol addendum be made, the informed consent form may be revised to reflect the changes of the protocol. If the consent form is revised, it is the responsibility of the investigator to ensure that an amended consent form is reviewed and approved by the IRB. The approved amended consent form should be signed by all subjects subsequently entered in the study and those currently in the study. The investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The terms of the consent and when it was obtained must be documented.

The informed consent form will contain the following information:

- A statement that the study involves research;
- An explanation of the purposes of the research;
- The expected duration of the subject's participation;
- A description of the procedures to be followed;
- Identification of and information about the active study drug, sacrosidase;
- A description of any reasonably foreseeable risks or discomforts to the subject;
- A description of any benefits to the subject or to others which may reasonably be expected from the research;
- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject;
- A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained;

- Research, rights, or injury: An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject; and
- A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

13.4. Protocol Deviations

Deviations from the protocol should not occur. If deviations occur, implications of the deviation must be reviewed by the investigator. Any deviation must be documented, stating the reason and date, the action taken, and the impact for the subject and/or the trial. All protocol deviations will be reported and accounted for in the statistical analysis.

14. DATA HANDLING AND RECORD KEEPING

14.1. Data Collection and Management Responsibilities

The study outcomes will be documented on subjected-reported Daily Symptom Questionnaires and Dosing Logs that will be completed either electronically or on paper. The integrity and reproducibility of these questionnaires is essential. When completing questionnaires on paper, subjects will be instructed to complete the questionnaires in a neat, legible manner to ensure an accurate interpretation of data. The use of a black ink pen will be required to ensure clarity of reproduced copies. The subjects will be instructed that when making changes or corrections, they are to cross out the original entry with a single line, and initial and date the change. They will be told to not erase, overwrite, or use correction fluid or tape on the original questionnaire.

The subject-reported questionnaires will be completed during the following periods:

- Baseline Period;
- Treatment Period 1;
- Washout Period; and
- Treatment Period 2.

The completed questionnaires will be collected during clinic visits 2-5. Data collection is the responsibility of the clinical trial staff at the study site, under the supervision of the study PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

The sponsor or designee will perform monitoring to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

14.2. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRFs unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- Guidance on completion will be provided.
- The investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source documents.
- Monitoring details will be described in the monitoring plan.
- The sponsor or designee is responsible for the data management of this study, including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., contract research organizations).

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16. APPENDICES

16.1. Appendix A: Sample Daily GI Symptom Questionnaire

Gastrointestinal Symptom Definitions

1. Abdominal Bloating

Abdominal or stomach bloating occurs when your stomach feels full and tight or looks larger than usual. Some people describe stomach bloating as the feeling that there is an inflated balloon in their belly. The bloating can cause your stomach to push out to the front, to the side, or both.

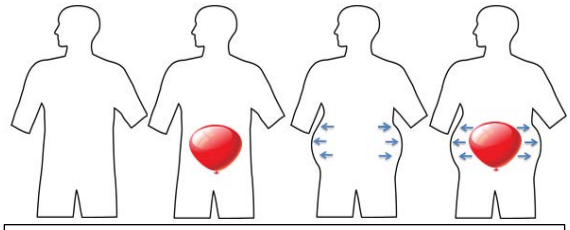

Figure 1: Stomach bloating/distension may feel like there is a balloon expanding in your stomach.

2. Abdominal Pain

Abdominal pain refers to pain anywhere above your belly button, around your belly button, or below your belly button. The area can be more sensitive and painful to the touch. Abdominal pain is sometimes referred to as a bellyache or a stomachache.

3. Abdominal Cramps

Abdominal cramps refer to a sudden, tight feeling in the muscles of your stomach.

4. Abdominal Gas/Flatulence

Flatulence is the act of expelling intestinal gas through your rectum. Other words for flatulence are farting, having gas, or passing wind. Belching and flatulence are very common. However, there may be times when you burp and/or fart more frequently than normal, becoming excessive and bothersome.

5. Diarrhea

Diarrhea refers to a stool that is abnormally loose (mushy) or watery. When you have diarrhea, you may also have more frequent bowel movements than normal.

6. Constipation

Constipation refers to a stool that is hard, dry, and lumpy. You may have a feeling that the stool is difficult or painful to pass or a feeling that you have not passed all the stool in your rectum.

7. Nausea

Nausea is feeling the urge to vomit. It is often called “being sick to your stomach.” You can feel nausea without actually vomiting.

Daily Symptom Questionnaire

Write down today's date, your subject ID number, and your initials at the top of each page. Please review the *Daily Symptoms – Overview and Practice* document before filling out this questionnaire.

Please record the number of episodes of each of these 7 gastrointestinal (GI) symptoms you experienced in the past 24 hours:

How many times did you experience abdominal bloating?					
0	1	2	3	4	5 or more
How many times did you experience abdominal pain?					
0	1	2	3	4	5 or more
How many times did you experience abdominal cramps?					
0	1	2	3	4	5 or more
How many times did you have abdominal gas/flatulence?					
0	1	2	3	4	5 or more
How many times did you have diarrhea?					
0	1	2	3	4	5 or more
How many times did have constipation?					
0	1	2	3	4	5 or more
How many times did you experience nausea?					
0	1	2	3	4	5 or more

Please rate the severity of these GI symptoms you experienced in the past 24 hours, on a scale of 0 to 9, where 0 = *No Symptoms*, and 9 = *Worst Symptom Severity*.

Abdominal Bloating									
0	1	2	3	4	5	6	7	8	9
Abdominal Pain									
0	1	2	3	4	5	6	7	8	9
Abdominal Cramps									
0	1	2	3	4	5	6	7	8	9
Abdominal Gas/Flatulence									
0	1	2	3	4	5	6	7	8	9
Diarrhea									
0	1	2	3	4	5	6	7	8	9
Constipation									
0	1	2	3	4	5	6	7	8	9
Nausea									
0	1	2	3	4	5	6	7	8	9

16.2. Appendix B: Sample Dosing Log

Sacrosidase Oral Solution Dosing Log

Please complete the date, time, and meal type of each sacrosidase dose during the 7-day treatment period.

Sacrosidase dosing instructions: Mix 2 mL using the measuring scoop (2 scoopfuls) with 4 ounces (120 mL) of cool to room temperature water or milk. Drink half of the mixture before eating each meal or snack and drink the remaining half of the mixture while eating each meal or snack up to 6 times per day.

Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)

	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
Date (MM/DD/YYYY)	Time of Dose (HH:MM)	Meal Type (Circle One)
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
______/_______/________	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack
	:	Breakfast Lunch Dinner Snack