A PHASE 1 RANDOMIZED SINGLE ORAL DOSE CROSS-OVER STUDY INVESTIGATING DESMETRAMADOL DOSE PROPORTIONALITY AND FOOD EFFECT IN NORMAL HUMAN SUBJECTS

Syntrix Protocol OMNI-PAIN-103

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Study Agent:	Desmetramadol 10 mg tablets
Protocol Number:	Syntrix-OMNI-PAIN-103
IND Number:	103,997
Study Site:	Celerion
Principal Investigator:	Robert Schwab, MD
Sponsor:	Syntrix Biosystems, Inc.
Personnel:	Stuart Kahn, MD (Project Director, Sponsor's Medical Monitor) Aaron Schuler, PhD (Formulation Scientist) John Zebala, MD, PhD (Regulatory Affairs)

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Principal Investigator Agreement

Protocol Number: Syntrix-Omni-Pain-103

I, the undersigned, have reviewed this protocol, including all attached information.

I agree to conduct this clinical study in accordance with the E6 Guidance of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) "Good Clinical Practice: Consolidated Guidance", and the U.S. Code of Federal Regulations governing the protection of human subjects (21 CFR 50), Institutional Review Boards (21 CFR 56) and the obligations of clinical investigators (21 CFR 312). Furthermore, I agree to maintain all study documentation for the time specified in this protocol.

_____

Principal Investigator Signature

_____

Printed Name

_____

Date

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List of Abbreviations

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
API	Active Pharmaceutical Ingredient
AUC	Area Under the Curve
AUMC	Area Under the First Moment Curve
BMI	Body Mass Index
BP	Blood Pressure
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CPU	Clinical Pharmacology Unit
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
Css_{max}	Concentration steady state maximum
Css_{min}	Concentration steady state minimum
CYP	Cytochrome P-450
DLDD	Drug Liking, Drug Disliking
DSM	Data and Safety Monitoring
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EM	Extensive metabolizers
EMA	European Medicines Agency
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBV	Hepatitis B Virus
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIPAA	Health Assurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HR	Heart Rate
HZ	Heterozygous
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference for the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IM	Intermediate metabolizer

IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-To Treat
IVIVC	In-Vitro In-Vivo correlation
LOQ	Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
MM	Medical Monitor
MOP	Manual of Procedures
M1	Metabolite 1
N	Number (typically refers to subjects)
NDA	New Drug Application
NE	Norepinephrine
NET	Norepinephrine Transporter
NIDA	National Institute On Drug Abuse
NIH	National Institutes of Health
O-DMT	O-Desmethyltramadol
OHRP	Office for Human Research Protection
OHSR	Office for Human Subjects Research
PACU	Post-Anesthetic Care Unit
PE	Physical Exam
PHI	Protected Health Information
PI	Principal Investigator
PID#	Participant Identification Number
PK	Pharmacokinetic
PM	Poor metabolizers
QA	Quality Assurance
QC	Quality Control
RR	Respiratory Rate
SAE	Serious Adverse Event
SID#	Screening Identification Number
SR	Slow release
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SRSDE	Subject-Rated Strength of Drug Effect
SSP	Study Specific Procedure
TDAA	Take Drug Again Assessment
TEA	Treatment Enjoyment Assessment
VAS	Visual Analogue Scale
UM	Ultra-rapid metabolizer
WBC	White Blood Cell
WHO	World Health Organization

Protocol Summary (Synopsis)

Protocol Number: OMNI-PAIN-103

Clinical Trial Phase: Phase 1

Sponsor: Syntrix Biosystems, Inc.

IND Sponsor: Syntrix Biosystems, Inc.

Objectives:

The purpose of the study is to investigate in healthy human subjects desmetramadol dose-proportionality and food effect (desmetramadol is the M1 metabolite of tramadol). Dose proportionality will be assessed for 10, 20 and 30 mg single oral doses, and food effect will be assessed for the 30 mg single oral dose.

Primary Objectives:

To determine the dose proportionality of desmetramadol following oral single-dose administration of 10, 20 and 30 mg in fasted healthy subjects.

To determine the food effect on 30 mg desmetramadol in healthy subjects following oral single-dose administration.

To determine the safety and tolerability of desmetramadol following oral single-dose administration in fasted and fed healthy subjects.

Secondary Objectives:

Determine PK parameters for each M5 enantiomer in blood following oral single-dose administration of 10, 20 and 30 mg desmetramadol in fasted healthy subjects and of 30 mg desmetramadol administered with food.

Quantify total M1 and M5 excreted (unconjugated and de-conjungated) in the urine after the 30 mg fasted dosings and compute clearance of each and in relation to 24 hr creatinine clearance.

Study Population:

Potential study subjects are screened and consented. Subjects will be men and women aged 19 to 55 years in general good health.

Design:

An open-label, randomized, balanced, single-dose, four-treatment, four-period, four-sequence (using a Williams' square design) cross-over study with each dose separated by $\geq$3 days:

	Period			
Sequence	I	II	III	IV
1	30 mg, food	30 mg, fasted	10 mg, fasted	20 mg, fasted
2	10 mg, fasted	30 mg, food	20 mg, fasted	30 mg, fasted
3	20 mg, fasted	10 mg, fasted	30 mg, fasted	30 mg, food
4	30 mg, fasted	20 mg, fasted	30 mg, food	10 mg, fasted

To account for potential dropouts, up to 32 eligible subjects will be randomized to obtain a target sample of 24 subjects with PK responses at each of the four treatment periods (based on our completed phase 1 study in 43 subjects, dropouts are unlikely (~5%); see **Section 1.2.2**). Before each oral dose, subjects will be fasted overnight for at least 10 hours. Treatment sequences will include the following four unblinded single-dose oral treatments: 1) desmetramadol 1 x 10 mg tablet; 2) desmetramadol 2 x 10 mg tablets; 3) desmetramadol 3 x 10 mg tablets; and 4) desmetramadol 3 x 10 mg tablets following a high-fat, high-calorie breakfast served approximately 30 minutes before dosing and entirely consumed within 20 minutes. All subjects will fast for an additional four hours after desmetramadol administration. The fed treatment should be administered the desmetramadol dose approximately 30 minutes after the start of the meal. Desmetramadol will be administered with approximately 240 ml of water. No water is allowed one hour before and one hour after each desmetramadol administration.

This will be an inpatient study. Subjects will be admitted to the clinical pharmacology unit on Study Day -1, and administered a single oral dose treatment on Study Day 1, Study Day 4, Study Day 7 and Study Day 10. After completing study procedures on Day 11 the subject will be discharged from the facility.

Blood specimens for plasma preparation and PK analysis will be collected at the following times: pre-dose (0 h), and post-dose 0.5, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, and 32 h.

Urine will be collected as described in the **Appendix A Auxiliary** to determine the clearance of M1, M5 and creatinine and determine the fraction of the desmetramadol dose excreted as M1 and M5.

Other salient aspects of the approach to evaluating the investigational agent are enumerated below:

Sample Size and Randomization:

Up to 32 subjects will be randomized to obtain a target sample of 24 subjects with PK responses at each of the four treatment periods. Desmetramadol and tramadol are closely related chemicals and are therefore expected to have a similar intra-patient coefficient of variation and food-effect. Published studies indicate a ~15% intra-subject coefficient of variation for immediate release tramadol (Najib 2009, ESP_Pharma_Limited 2010, Dhanure 2013), and the Ultram label indicates that "oral administration of Ultram with food does not significantly affect its rate or extent of absorption, therefore, Ultram can be administered without regard to food."

Dose-proportionality will be established according to the power-model $Y_i = \alpha \cdot D_i^{\beta}$, where Y is the pharmacokinetic response (e.g., AUC, C_{max}), α is the coefficient, β the exponent, D the dose, and i the fasted dose levels to be explored (i.e., i = 1, 2 and 3 correspond to the 10, 20 and 30 mg doses, respectively). The power-model is linearized by natural log transforming the equation to $\ln(Y_i) = \alpha + \beta \cdot \ln(D_i)$. Strict dose linearity yields a slope β equal to 1.

Using a conservative estimate of 20% for the intra-subject coefficient of variation (CV) of desmetramadol, and a 5% level of significance, a sample size of 24 subjects is considered sufficient to provide 89.6% power (essentially 90% power) to establish dose-proportionality and absent food effect, assuming no multiplicity and a fed/fasting ratio of 0.95 and a 5% deviation from 1 for the slope β in dose proportionality. Based on a study drop-out rate of ~30% in the Ultram label, up to 8 subjects (alternates) might be added to maintain a sample of 24 subjects with blood draws at each treatment period (based on our completed phase 1 study in 43 subjects, dropouts are unlikely to exceed 5%; see **Section 1.2.2**).

Randomization of subjects will be balanced across the four treatment sequences in the 4x4 Williams' design.

Study Duration:

Enrollment is estimated to take approximately 30 days. Subjects will be on study for 11 Days. It will take approximately 6 weeks to complete the study after enrollment of the first subject.

Study Agent:

Test desmetramadol tablets: 10 mg slow release tablet.

Endpoints:

The endpoints are pharmacokinetic (PK) responses for all measured blood analytes (i.e., each enantiomer of M1 and M5): $T_{1/2}$, T_{max}, C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and if present, T_{lag}). The PK responses relevant to dose-proportionality and food effect are M1 $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max}.

The urine endpoints are CL_{M1}, CL_{M5}, CL_{CR}, and the mass of M1 and M5 excreted. Exploratory urine endpoints include calculating the rate constants for total elimination (k_{el}), renal elimination (k_e), and fraction excreted (f_e) by plotting the rate of excretion ($\Delta U/\Delta t$) vs. the midpoint time of each interval.

Safety: Adverse events.

Monitoring:

Syntrix Biosystems and its representatives.

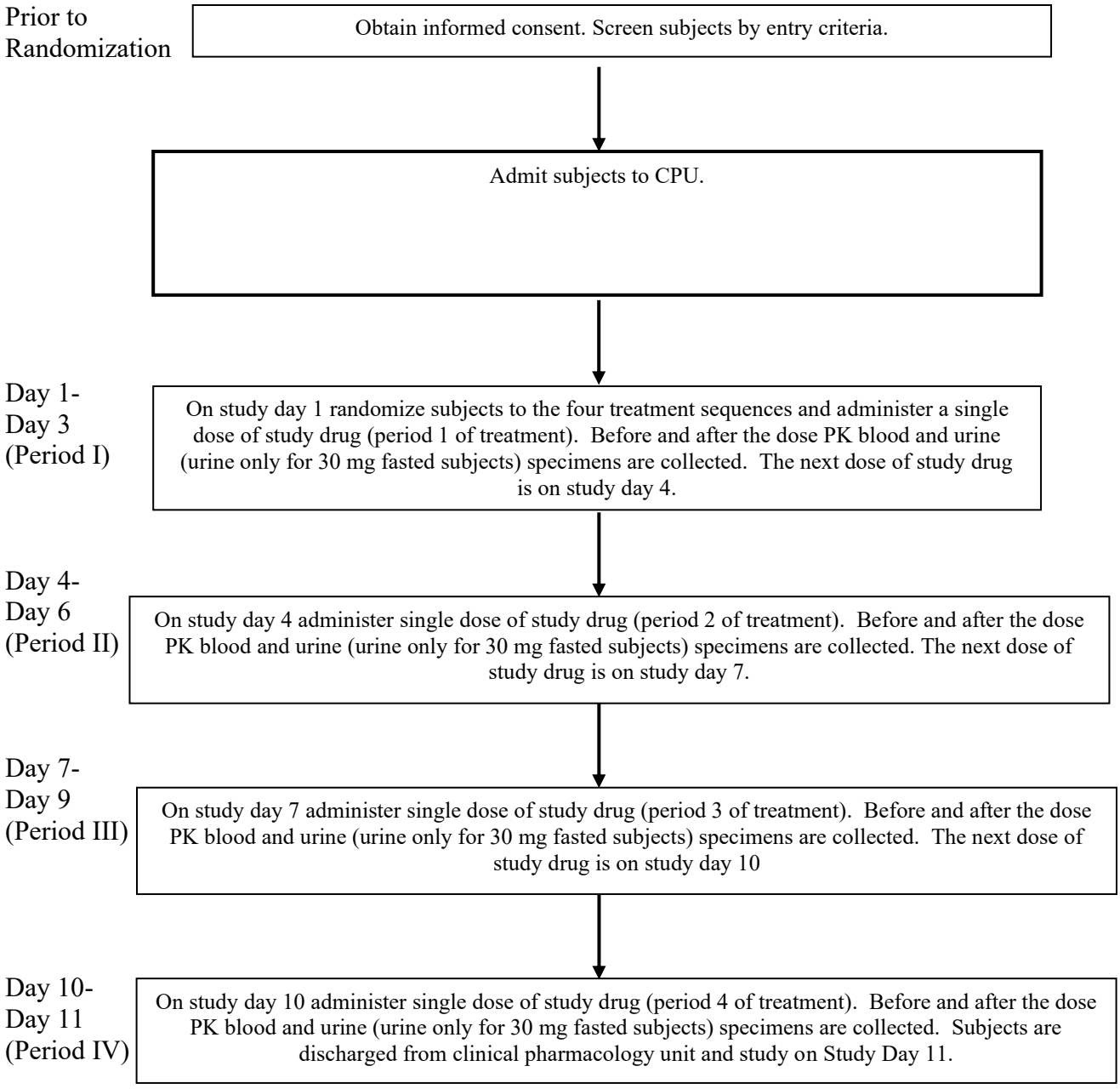
Clinical Site:

Celerion

Bioanalytical Laboratory:

Celerion

Schematic of Study Design



1 BACKGROUND AND SCIENTIFIC RATIONALE

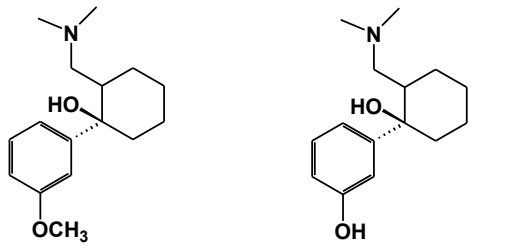
1.1 Background Information

1.1.1 Tramadol and desmetramadol: Background and Pharmacology

Tramadol is a synthetic atypical, centrally-acting opioid analgesic approved in the U.S. and elsewhere for treating moderate to severe pain with efficacy and potency between weak opioids and potent opioids such as morphine (Grond 2004). Tramadol was developed by the German pharmaceutical company Grünenthal GmbH in the late 1970s under the trade name Tramal. One of the advantages of tramadol over traditional opioids is its lower risk of opioid dependence (Raffa 2008). Tramadol is a widely prescribed generic analgesic either alone, or together with acetaminophen, with over 25 million prescriptions in the U.S. in 2009 (http://drugtopics.modernmedicine.com).

Tramadol is a racemate consisting of 1R, 2R-tramadol [(+)-tramadol], and 1S, 2S-tramadol [(−)-tramadol]. After oral administration of the racemate, both the (−) and (+) forms of both tramadol and the O-desmethyltramadol metabolite (O-DMT or M1) are detected in the circulation (**FIG. 1A**). desmetramadol, the test study agent, is racemic O-desmethyltramadol, and thus provides (-) and (+) O-DMT without requiring metabolism.

(**A**)



Tramadol O-desmethyltramadol (M1)

(**B**)

Compound	K_i (μM) opioid receptor affinity			uptake inhibition	
	μ	δ	κ	norepinephrine	serotonin
morphine	0.00034	0.092	0.57	inactive	inactive
(+)-tramadol	1.3	62.4	54.0	2.51	0.53
(-)-tramadol	24.8	213	53.5	0.43	2.35
(+)-O-DMT	0.0034	-	-	11-14.4	2.98
(-)-O-DMT	0.240	-	-	0.38-0.86	17.7

FIG. 1. Structures of (**A**) tramadol and the active metabolite O-DMT; and (**B**) relative inhibition of opioid receptors and monoamine transporters. Determined and estimated from (Grond 2004, Raffa 2012). Highlighted values reflect dominant target of compound based on affinity. [7].

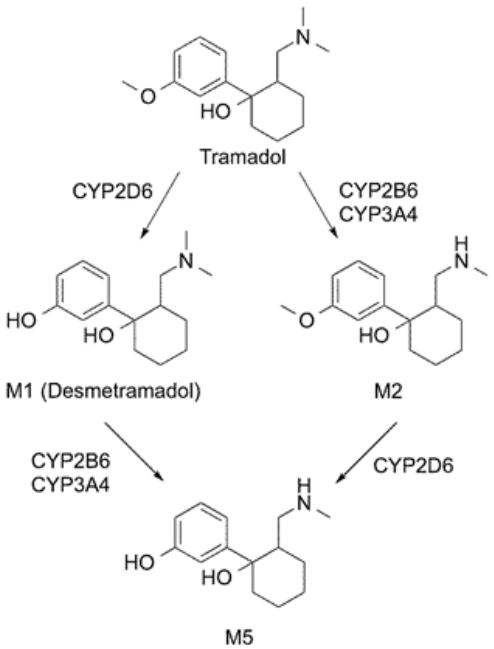
Each enantiomer of tramadol and O-DMT has a different dominant receptor or transporter target (**FIG. 1B**). These targets include (+)-O-DMT enantiomer binding to the μ-opioid receptor, and (-)-tramadol and (-)-O-DMT enantiomers binding to norepinephrine transporter (NET) (Kayser 1991, Kayser 1992, Raffa 1992, Driessen 1993, Desmeules 1996). The (-)-enantiomers of tramadol and O-DMT have substantially equivalent in vitro potency with respect to binding the NET and inhibiting NE reuptake.

Two synergistic mechanisms are believed involved in tramadol and O-DMT mediated analgesia. First, supraspinal μ-opioid receptor agonism by (+)-O-DMT increases serotonin and NE in the dorsal horn of the spinal cord via descending pathways originating from the locus coeruleus and possibly other areas (Ide 2006). Second, the released spinal NE is potentiated by the (-)-tramadol and (-)-O-DMT enantiomers blockade at the NET. The accumulated NE exerts antinociceptive action by reducing neurotransmitter release from primary afferent nerves.

In addition to the pharmacology of (+)-O-DMT, (-)-O-DMT, and (-)-tramadol, the (+)-enantiomer of tramadol directly inhibits serotonin reuptake (**FIG. 1B**) (Driessen 1992, Bamigbade 1997). Studies conducted in the μ-opioid receptor knockout mouse demonstrated that serotonin reuptake inhibition is not involved in tramadol analgesia (Ide 2006). Furthermore, the tramadol (+)-enantiomer inhibition of serotonin reuptake may trigger the serotonin syndrome, a rare potentially fatal known adverse reaction of tramadol. The serotonin syndrome is observed more frequently when tramadol is taken with other serotonin reuptake inhibitors, e.g., antidepressants (Sansone 2009). Thus, compared to tramadol, O-DMT has equivalent analgesia, but substantially less serotonin reuptake inhibition than tramadol (**FIG. 1B**) (Ide 2006, Yanarates 2010). Therefore O-DMT (desmetramadol), compared to tramadol, should offer equal analgesia and, through its decreased propensity to induce the serotonin syndrome, both improved safety and improved drug-drug compatibility.

Tramadol has 75% oral bioavailability, with peak serum concentrations reached within 2 hours (Grond 2004). Tramadol elimination kinetics are two-compartmental; after a single 100 mg oral dose of tramadol, tramadol half-life is 5.1 hours and 9 hours for O-DMT. The FDA approved dose of tramadol is 50-100 mg every 4 to 6 hours, with a maximum dose of 400 mg/day; the duration of the analgesic effect after a single oral dose of tramadol 100 mg is about 6 hours.

Tramadol is metabolized as follows, and desmetramadol is not expected to yield M2, but only M5:



1.1.2 Unmet Need: Absent Tramadol Efficacy in Patients with Low CYP2D6 Activity

Most individuals rapidly and extensively metabolize tramadol in the liver principally by the cytochrome P-450 (CYP) isoenzymes CYP2D6 and CYP2B6 (O-desmethylation), and CYP3A4 (N-desmethylation). Importantly, following tramadol administration O-DMT generation is dependent on CYP2D6. Approximately 7-10% of Caucasians have two inactive CYP2D6 alleles, and no or severely reduced CYP2D6 activity. These individuals are poor metabolizers (PMs) of tramadol, and following tramadol administration their M1 serum concentration is below the therapeutic range (50-100 ng/mL) after a 100 mg oral (Kirchheiner 2008) or iv dose (Enggaard 2006), being almost undetectable or below the limit of detection compared to extensive metabolizers (EMs) with two normal alleles, and confirming the dominant role of CYP2D6 in O-demethylation of tramadol in humans (**FIG. 2A**).

In addition to genetic deficiency of CYP2D6, studies have shown that patients with one or two normal CYP2D6 may be functionally deficient as a result of concurrent use of a drug that inhibits CYP2D6. These patients are said to be "phenoconverted" to poor metabolizers. Such findings are clinically important to the relevance of desmetramadol, because *multiple medication use is common in clinical practice*, and increases the risk of CYP2D6 phenoconversion and non-response to tramadol. Phenoconversion is *unpredictable* and is *not* detected by genetic testing. In one study of a university hospital medication database in Finland, every fifth patient receiving tramadol was concomitantly taking another drug that had the potential to inhibit its activation to O-DMT (Tirkkonen 2004). In another large-scale study that assessed the incidence of phenoconversion to CYP2D6 poor metabolizer status in patients being treated for depression in clinical practice in the U.S. (Preskorn 2013), the rate of phenoconversion was 27%. The incidence of CYP2D6 poor metabolizer status based on phenotype was ~7 times larger than that expected based on genotype.

Consistent with O-DMT being critical to mediating tramadol analgesia, several human studies have shown that tramadol efficacy is significantly decreased or lacking in PM patients. In the first study, using two parallel, randomized, double-blind, placebo-controlled crossover designs, the analgesic effect of tramadol was assessed in 27 volunteers (15 EMs and 12 PMs) using several experimental pain models (Poulsen 1996). Sufficient differences were noted between the EMs and the PMs to indicate that O-DMT is critical for the analgesic effect of tramadol on experimental pain.

In the second study, the effect of CYP2D6 polymorphism on tramadol analgesia was assessed in 300 Caucasian patients undergoing major abdominal surgery (Stamer 2003). Patients who had one or more functional alleles were classified as EMs. Genotyping revealed that 35 patients were PMs. Compared to the EMs, the PMs displayed a significantly higher incidence of non-response ($P = 0.005$) and frequently required rescue medication ($P = 0.02$).

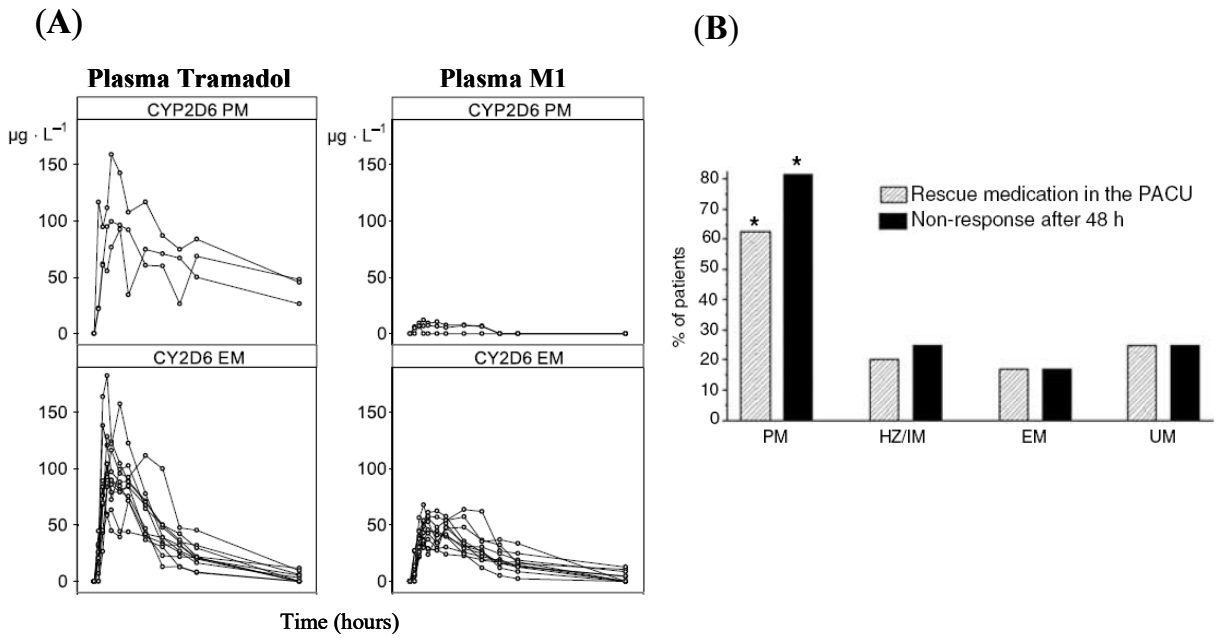


FIG. 2 (**A**) Tramadol and O-DMT plasma concentrations over time in subjects with different CYP2D6 genotypes; PM vs. EM. All subjects received 100 mg dose of tramadol po. (**B**) Non-responders to tramadol treatment allocated to genotypes. Grey columns: % of patients needing rescue medication in the post-anesthetic care unit (PACU); black columns: % of non-responders after the 48-h study period. *P<0.001 for the PM group compared with the HZ/IM, EM, and UM groups.

In the third study, the effect of CYP2D6 polymorphism (specifically CYP2D6*10, a SNP that results in a Pro34 to Ser substitution and reduced CYP2D6 activity) on tramadol-induced analgesia was assessed in 63 Chinese patients who underwent gastrectomy for gastric cancer (Wang 2006). The patients were classified as EMs (N = 17), or heterozygous (N = 26) or homozygous (n = 20) for CYP2D6*10. Compared to the other groups, the homozygous group required more tramadol (P<0.05).

Finally, a fourth study of patients (N=187) undergoing major abdominal surgery reported a 4-fold greater non-response rate to tramadol in PMs (Stamer 2007); (**FIG. 2B**).

In summary, the analgesic effect of tramadol is decreased or absent in patients with low CYP2D6 enzymatic activity (PMs) because their serum O-DMT concentration is considerably lower.

1.1.3 Significant Negative Impact on Patient Care and Safety

Tramadol is widely prescribed, with over 25 million prescriptions in the U.S. in 2009 (http://drugtopics.modernmedicine.com). As such, 10-30% drug resistance in a target patient population due to CYP2D6 PM phenotype results in significant negative impact on patient care. Based on an estimated 3:1 prescription-to-patient ratio, these data suggest that upwards of 1-2 million patients annually may receive inadequate analgesia from tramadol therapy. Furthermore, the need to switch non-responders to traditional opioids increases their risk of opioid dependence.

Thus, there exists a need to develop an “improved tramadol” (desmetramadol (O-DMT)) that would be effective in all patients irrespective of their metabolic status, and safer. desmetramadol could serve all patients being treated with tramadol, as well as a significant portion of patients who are being treated with conventional opioids that are plagued with similar issues of metabolic variability.

1.2 Description of the Study Agent

1.2.1 Pilot Study of Desmetramadol in a Human Subject

1.2.1.1 Single Dose Pharmacokinetics

Single-dose pharmacokinetics of tramadol and desmetramadol are shown in **FIG. 3**. In the Hagelberg study, 12 healthy subjects were randomized in three segments to ingest 50 mg of tramadol after 4 days of pretreatment with either placebo, ticlopidine (250 mg twice daily) or ticlopidine plus itraconazole (200 mg once daily). Data for the M1 exposure in the placebo treated subjects is shown. M1 exposure for a single 100 mg tramadol dose is from the Ultram label and the Grond review. The pharmacokinetic parameters for M1 exposure from 20 mg desmetramadol approximates those for 50 mg tramadol.

Drug	**tramadol**	**tramadol**	**desmetramadol (M1)**
Study	(Hagelberg 2013)	Ultram label, (Grond 2004)	(SYN-PAIN-115 2011)
n	12	12	1
population	healthy adult	healthy adult	healthy adult
Route	oral	oral	oral
Dose (mg)	50	100	20
Fasting	Fasted then meal at 4 and 8 hours	"food does not significantly affect its rate"	Fasted 30 min before and after dosing
M1 Exposure			
AUC (ng-h)/mL	367	722	229
C_{max} (ng/mL)	34	55.0	28.8
T_{max}	2.7	3.0	3.6
Half-life (hr)	6.6	6.7	6.0

FIG. 3. Single-dose PK parameters for M1 exposure in man from 50 and 100 mg tramadol and 20 mg desmetramadol.

1.2.1.2 Steady-State Pharmacokinetics

The approved oral dosage of tramadol is 50-100 mg q 6 hours, and the mean steady-state level of M1 produced by this approved dosage is ~55-110 ng/mL (see Ultram label). This is the same steady-state M1 level produced by 20-30 mg oral desmetramadol q 6 hours (i.e., 2-3 10 mg desmetramadol tablets q 6 hours) (**FIG. 4**). Importantly however, desmetramadol achieves therapeutic M1 levels without requiring metabolism by CYP2D6, thus obviating the shortcomings of tramadol related to CYP2D6.

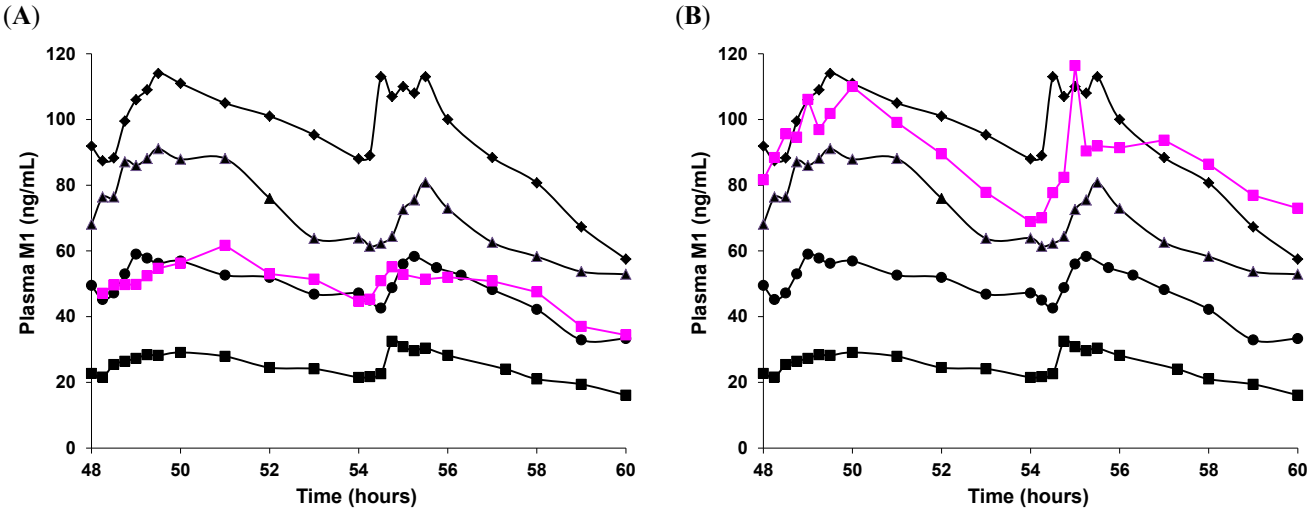


FIG. 4. Steady-state data for tramadol and desmetramadol in a healthy male human subject. Black traces in (**A**) and (**B**) are dose 9 (48-54 hours) and 10 (54-60 hours) of tramadol q 6 hours: 25 mg (squares), 50 mg (circles), 75 mg (triangles), 100 mg (diamonds). Purple traces represent dose 9 and 10 of desmetramadol (10 mg tablet) q 6 hours: 20 mg (**A**) and 30 mg (**B**).

1.2.2 Phase 1 PK and PD Study in Normal Healthy Volunteers

In October 2014 the Sponsor completed a phase 1 trial entitled "A Phase 1 Randomized, Double-Blind, Placebo-Controlled, Double Cross-Over Study Investigating the Safety, Oral Steady-State Pharmacokinetics, and Clinical Activity of 20 Mg desmetramadol and 50 Mg Tramadol in Normal Human Subjects". Briefly, 42 males in normal health, 21 to 55 years of age, were inpatients for 3 days at a clinical pharmacology unit and randomized to three parallel arms (N=14 each) to ingest a total of 9 blinded doses of desmetramadol (two 10 mg tablets), tramadol (one 50 mg tablet), or placebo in a first treatment segment (one dose every 6 hours). Immediately before the 9th dose, and at times (1.0, 1.5, 2.0, 2.5 and 4.0 hours) after the 9th dose blood samples were collected to quantify plasma M1 and tramadol enantiomers to identify Css_{min} and Css_{max}. Pain tolerance was assessed in the cold pressor test at three sequential time points (1.0, 2.0, and 3.0 hours) after the 9th dose and averaged to provide a single value for subsequent analyses. The cold pressor test involved non-dominant hand immersion in ice-cold water (2 °C ± 0.5°C) for up to 3 minutes, an established experimental pain measure used in previous studies of tramadol (Poulsen 1996, Hagelberg 2013). Measurements included (a) time to first pain perception, (b) pain intensity at first pain perception, (c) pain at 30 seconds, (d) pain at 60 seconds, (e) total time immersed not to exceed 3 minutes. Subjects were discharged from the clinical pharmacology unit after the last blood draw and washed out for 7 days (i.e., after the first and second treatment segments) before being readmitted. The endpoint analyses included the steady-state parameters Css_{min} and Css_{max}, pain tolerance, and safety.

The PK equivalence of desmetramadol to tramadol was evaluated using the log-transformed Css_{min} and Css_{max} of M1 (note only two genetic CYP2D6 PMs were in the population). Formal bioequivalence is claimed for a Css parameter when the 90% confidence interval of its desmetramadol-to-tramadol ratio falls within 0.8 and 1.25 (Rani 2007). Pain tolerance for desmetramadol, tramadol and placebo were compared using a mixed effects linear model with treatment, period, and sequence as fixed effects and subject as a random effect nested within sequence. Period was added as a fixed effect to find any significant first-order crossover effects. If significant treatment effects were present, least squares means were compared between desmetramadol and placebo treating placebo as a control using the Dunnett's procedure.

Steady-state dosing of 20 mg desmetramadol was formally bioequivalent to 50 mg tramadol with respect to plasma (+)-O-desmethyltramadol (**FIG. 5A**). desmetramadol was nearly formally bioequivalent with respect to (-)-O-desmethyltramadol: Css_{min} (21$\pm$5 v. 30$\pm$6 ng/mL) and Css_{max} (30$\pm$9 v. 42$\pm$9 ng/mL). This Phase 1b trial confirmed the earlier pilot data, and showed desmetramadol indeed successfully "flattened" the steady-state blood levels of M1 between doses in a manner equivalent to that obtained with tramadol (**FIG. 5B**). Twenty mg desmetramadol is therefore a therapeutic substitute for 50 mg tramadol with respect to the steady-state delivery of M1, a finding that informed the desmetramadol dose selected for this phase 1 trial to examine the effect phenoconversion of CYP2D6 on desmetramadol and tramadol analgesia in normal subjects.

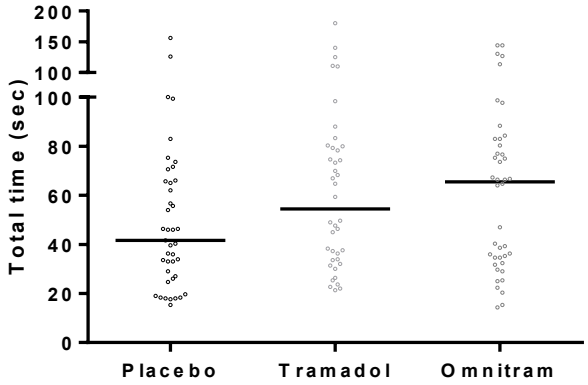
Evaluation of total time immersed in the cold pressor test revealed a significant difference between desmetramadol vs. placebo ($P = 0.0060$) and tramadol vs. placebo ($P = 0.0076$) (**FIG. 5C**). A significant difference was also found between desmetramadol and placebo for time to first pain perception ($P = 0.0057$), and pain at 30 seconds ($P = 0.0019$). Data was lacking for pain at 60 seconds due to hand withdrawal before 60 seconds. The adverse events after desmetramadol and tramadol were similar (**FIG. 5D**).

(A)

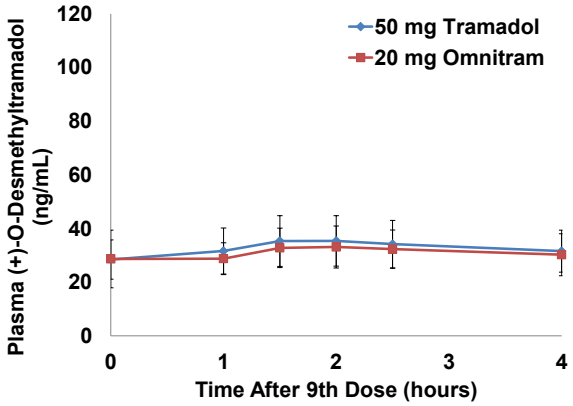
Treatment	Css_{min} ng/mL	Css_{max} ng/mL	Css_{mean} ng/mL
20 mg desmetramadol (test)	26 ± 6	36 ± 10	31 ± 7
50 mg Ultram IR (reference)	28 ± 7	37 ± 10	33 ± 8
90% CI of ratio	0.85-1.08*	0.88-1.13*	-

*90% CI within 0.80-1.25; formally bioequivalent. Mean±SD.

(C)



(B)



(D)

Preferred Term	desmetramadol (n=43)	tramadol (n=41)	placebo (n=41)
Dizziness	5 (12%)	5 (12%)	1 (2%)
Headache	3 (7%)	6 (15%)	0 (0%)
Nausea	8 (19%)	5 (12%)	3 (7%)
Pruritus	0 (0%)	1 (2%)	0 (0%)
Pruritus generalized	3 (7%)	3 (7%)	0 (0%)
Somnolence	3 (7%)	2 (5%)	0 (0%)
Urinary hesitation	1 (2%)	2 (5%)	0 (0%)
Vomiting	1 (2%)	1 (2%)	0 (0%)

FIG. 5. Summary of results from Phase 1b trial. (**A**) tabular and (**B**) graphical summary (mean±SD) of steady-state plasma +M1 due to 20 mg desmetramadol q6 hr and 50 mg tramadol q6 hr. (**C**) Graphical summary total immersion time in cold pressor test as a function of treatment with placebo, tramadol and desmetramadol (horizontal bar is median). The mixed effects linear model was applied to the data. The Type III tests of fixed effects showed a significant treatment effect ($P = 0.0036$) and no significant sequence or period effects. Least square means for each treatment showed that desmetramadol had the highest mean (62.8) with placebo having the lowest mean (50.0). After observing a significant treatment effect, Dunnett's procedure was run to find which study treatments were significantly different than the control (placebo). Dunnett's procedure indicated a significant difference between both desmetramadol vs. placebo ($P = 0.0060$) and tramadol vs. placebo ($P = 0.0076$). (**D**) Tabular summary of adverse events.

1.3 Rationale

1.3.1 Overall Study Design

For this study, Syntrix Biosystems, through its CMO, has manufactured desmetramadol 10 mg SR tablets (10 mg SR O-DMT) to be the test drug. This study will investigate in male and female subjects single-dose oral PK responses of desmetramadol (test drug) following administration: in a fasted state of 10 mg (1x10 mg tablet); 20 mg (2x10 mg tablets); and 30 mg (3x10 mg tablets); and in a fed state: 30 mg (3x10 mg tablets).

This protocol is designed to perform a randomized, open-label, four sequence, four period, cross-over study to analyze the fasted-state oral dose proportionality of desmetramadol, and the effect of food on desmetramadol.

1.3.2 Dosage

The rationale for the efficacy and safety of the 10 mg, 20 mg, and 30 mg single dose desmetramadol SR is based on 20 mg desmetramadol providing a similar M1 C_{max} as does the FDA-approved single dose of 50 mg tramadol, and 20 mg desmetramadol providing the same steady-state M1 exposure as does the FDA-approved dosing of 50 mg tramadol every 6 hours. The extensive public database on the safety and adverse events associated with the use of tramadol at the approved dosing of 50-100 mg q 6 hours defines the safety profile of 10 mg, 20 mg, and 30 mg of desmetramadol SR.

1.3.2.1 Nonclinical Toxicology Data

See the Investigator's Brochure.

1.3.2.2 Clinical Data

See the Investigator's Brochure.

1.3.3 Study Population

For this phase 1 study, the study population is male and female volunteers in normal health 19 to 55 years of age.

1.3.4 Study Endpoints

The endpoints are pharmacokinetic (PK) responses for all measured blood analytes (i.e., each enantiomer of M1 and M5): $T_{1/2}$, T_{max}, C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and if present, T_{lag}). The PK responses relevant to dose-proportionality and food effect are M1 $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max}.

The urine endpoints are CL_{M1}, CL_{M5}, CL_{CR}, and the mass of M1 and M5 excreted. Exploratory urine endpoints include calculating the rate constants for total elimination (k_{el}), renal elimination (k_e), and fraction excreted (f_e) by plotting the rate of excretion ($\Delta U/\Delta t$) vs. the midpoint time of each interval.

Safety endpoints are adverse events.

1.4 Potential Risks and Benefits

Significant risks or benefits are not expected. Formally, however, potential physical risks include side-effects commonly known to be associated with tramadol. The side-effects from the test drug desmetramadol are not expected to be qualitatively or quantitatively worse than the side-effects arising from tramadol. Additional risks associated with any pharmacokinetic study are also possible including minor bruising and hematoma at the site of vein puncture.

The eventual availability of desmetramadol for pain will be beneficial as an alternative to tramadol as it will result in fewer analgesic non-responders.

2 STUDY OBJECTIVES

The purpose of the study is to investigate in healthy human subjects desmetramadol dose-proportionality and food effect (desmetramadol is the M1 metabolite of tramadol). Dose proportionality will be assessed for 10, 20, and 30 mg single oral doses, and food effect will be assessed for the 30 mg single oral dose. The primary objectives are to:

- Determine the dose proportionality of desmetramadol following oral single-dose administration of 10, 20, and 30 mg in fasted healthy subjects.
- Determine the food effect on 30 mg desmetramadol in healthy subjects following oral single-dose administration.
- Determine the safety and tolerability of desmetramadol following oral single-dose administration in fasted and fed healthy subjects.
- Determine PK parameters for each M5 enantiomer in blood following oral single-dose administration of 10, 20 and 30 mg desmetramadol in fasted healthy subjects and of 30 mg desmetramadol administered with food.
- Quantify total M1 and M5 excreted (unconjugated and de-conjungated) in the urine after the 30 mg fasted dosings and compute clearance of each and in relation to 24 hr creatinine clearance.

3 STUDY DESIGN

3.1 Description of the Study Design

An open-label, randomized, balanced, oral single-dose, four-treatment, four-period, four-sequence (using a Williams' square design) cross-over study as follows:

	Period			
Sequence	I	II	III	IV
1	30 mg, food	30 mg, fasted	10 mg, fasted	20 mg, fasted
2	10 mg, fasted	30 mg, food	20 mg, fasted	30 mg, fasted
3	20 mg, fasted	10 mg, fasted	30 mg, fasted	30 mg, food
4	30 mg, fasted	20 mg, fasted	30 mg, food	10 mg, fasted

To account for potential dropouts, up to 32 male and female subjects, 19 to 55 years of age, could be randomized to obtain a target sample of 24 subjects with PK responses at each of the four treatment periods (based on our completed phase 1 study in 43 subjects, dropouts are unlikely; see **Section 1.2.2**). Before each oral dose, subjects will be fasted overnight for at least 10 hours. Treatment sequences will include the following 4 unblinded single-dose oral treatments: 1) desmetramadol 1 x 10 mg tablet; 2) desmetramadol 2 x 10 mg tablets; 3) desmetramadol 3 x 10 mg tablets; and 4) desmetramadol 3 x 10 mg tablets following a high-fat, high-calorie breakfast served approximately 30 minutes before dosing and that is to be entirely consumed within 30 minutes. Each dose will be separated by 3 days. All subjects will fast for an additional four hours after desmetramadol administration. The fed treatment should be

administered and the desmetramadol dose administered approximately 30 minutes after the start of the meal. Desmetramadol will be administered with approximately 240 ml of water. Only water is allowed one hour before and one hour after each desmetramadol administration.

This will be an in-patient study. Subjects will be admitted to the clinical pharmacology unit on Study Day -1, and administered a single oral dose treatment on Study Day 1, Study Day 4, Study Day 7 and Study Day 10. After completing study procedures on Day 11 the subject will be discharged from the facility.

Blood specimens for plasma preparation and PK analysis will be collected at the following times: pre-dose (0 h), and post-dose 0.5, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 24, and 32 h.

Urine will be collected as described in the **Appendix A Auxiliary** to determine the clearance of M1, M5 and creatinine and determine the fraction of the desmetramadol dose excreted as M1 and M5.

Safety will be assessed while subjects are inpatients for each of the four treatment segments.

After the study drug dosing and evaluations are completed the subjects will be discharged from the clinical pharmacology unit.

Blood will be obtained for: 1) safety assessment at screening and on Day 11; and 2) for desmetramadol PK assessment on Days 1, 2, 4, 5, 7, 8, 10, and 11.

3.2 Study Endpoints

3.2.1 Primary Endpoint-Pharmacokinetic Responses

The primary endpoints are pharmacokinetic (PK) responses for all measure blood analytes (i.e., each enantiomer of M1 and M5): $T_{1/2}$, T_{max}, C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and if present, T_{lag}). The PK responses relevant to dose-proportionality and food effect are M1 $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max}.

The urine endpoints are CL_{M1}, CL_{M5}, CL_{CR}, and the mass of M1 and M5 excreted. Exploratory urine endpoints include calculating the rate constants for total elimination (k_{el}), renal elimination (k_e), and fraction excreted (f_e) by plotting the rate of excretion ($\Delta U/\Delta t$) vs. the midpoint time of each interval.

The PK parameters will be determined from the desmetramadol concentrations in the blood and urine samples collected before and after each study drug dose. To determine dose proportionality, results will be compared between the three fasted treatment groups (fasted desmetramadol: 10 mg, 20 mg, and 30 mg). To determine food effect, results will be compared between the two 30 mg dose treatment groups (fasted and fed).

3.2.2 Secondary Endpoint-Safety

Any abnormal laboratory values, abnormal vital signs, reported symptoms, or abnormal physical examination findings determined to be clinically significant by the Investigator/designee will be documented as adverse events. The safety assessments will be based on all reported adverse events, and changes in laboratory values from baseline. The severity and relationship to desmetramadol treatment

will be recorded for all adverse events. Adverse events will be coded for summary and analysis using standardized preferred terms and system organ class.

3.3 Study Site

This trial will be conducted at a clinical pharmacology unit. Study products will be stored at the site under controlled conditions.

4 STUDY POPULATION

The study population is male and female subjects in normal health to be evaluated at the clinical pharmacology unit. A subject is considered enrolled in the study once they have completed the informed consent, completed the screening assessments, found to be eligible, and have been randomized. The goal is to enroll sufficient subjects so that 24 subjects complete the study with PK specimens collected for each period. The subjects will be randomized on Day 1 to one of four sequences, with each sequence having four treatments (e.g., after fasting either 10 mg, 20 mg, 30 mg desmetramadol; or after a high fat meal, 30 mg desmetramadol). The study population is defined by all randomized subjects.

Children and subjects less than 19 years of age will be excluded from this phase 1 study. The study will not enroll children, pregnant women, prisoners, or other vulnerable populations. The study is therefore exempt from 45 CFR 46 Subparts B, C, and D.

The study population will include all subjects who have been randomized. The safety population will include all subjects who have been randomized and have received study drug. The pharmacokinetic population will be defined in the SAP.

4.1 Subject Inclusion Criteria

During screening, subjects must meet all of the following inclusion criteria to participate in this study:

1. Healthy males and females with vital signs as follows at screening: systolic blood pressure $\geq$ 90 mm Hg and $\leq$ 140 mm Hg; diastolic blood pressure $\geq$ 40 mm Hg and $\leq$ 90 mm Hg; pulse 40 to 99 beats per minute; respiratory rate 12 to 24 breathes per minute.

2. Age 19 to 55 years.

3. Able and willing to give informed consent

4. Able to comply with all study procedures.

5. If female, must not be of childbearing potential or must agree to use one or more of the following forms of contraception from screening, throughout the study and for 30 days following study drug administration: hormonal (e.g., oral, transdermal, intravaginal, implant or injection for 3 months); double barrier (e.g., condom or diaphragm with spermicide); intrauterine device (IUD) or system (IUS) (for 3 months); vasectomized partner (6 months minimum); or abstinence.

   Screening laboratory results must be within normal range, or judged by the PI to be not clinically significant: serum sodium, potassium, calcium, BUN, creatinine, ALT, AST, total bilirubin, alkaline

phosphatase, glucose (random), albumin, total protein, WBC and differential, hemoglobin, and platelets. In addition PT and PTT must be < 1.2 ULN. Urinalysis demonstrating ≤ +1 glucose, and +1 protein.

6. Electrocardiogram (ECG) without clinically significant abnormalities.

7. If female, must have a negative pregnancy test at screening and when performed on Day -1 (**Appendix A**).

8. Negative urine test for substances of abuse, including opiates, per clinical pharmacology unit standards at screening and clinic check-in.

9. Negative serology tests for HIV, hepatitis B surface antigen, and hepatitis C virus antibody.

10. Weight ≥ 50 Kg and a body mass index (BMI) of 18.0 to 32.0 kg/m (inclusive).

11. Non-smoker of tobacco for a minimum of the past 3 months, and negative urine continine test.

4.2 Subject Exclusion Criteria

During screening, subjects meeting any of the following exclusion criteria will be excluded from study participation:

1. Oral temperature > 38°C or current illness.

2. History of seizures, epilepsy, or recognized increase risk of seizure (e.g., head trauma, metabolic disorders, alcohol or drug withdrawal).

3. History of cirrhosis or laboratory evidence of liver disease.

4. Having undergone gall bladder removal.

5. Use of alcohol within 24 hours of day -1 until the end of the study; and grapefruit, grapefruit-related citrus fruits (e.g., Seville oranges, pomelos), or grapefruit juice or grapefruit-related juices within 7 days of study drug administration and until the end of the study.

6. History of previous anaphylaxis, severe allergic reaction to tramadol, codeine, or other opioid drugs.

7. Any other unstable acute or chronic disease that could interfere with the evaluation of the safety of the study drug as determined by the principal Investigator. If the appropriateness of a participants enrollment is unclear the the Sponsor's Medical Monitor can be consulted.

8. Females must not be currently pregnant or breast feeding.

9. Unlikely to comply with the study protocol.

10. Known or suspected alcohol or drug abuse within the past 6 months.

11. Received another investigational agent within 4 weeks of Day -1, or within five half-lives of Day -1, whichever is longer; or receiving any other investigational agent during this study.

12. Any concurrent disease or condition that in the opinion of the investigator impairs the subject's ability to complete the trial. Psychological, familial, sociological, geographical or medical conditions which, in the Investigator's opinion, could compromise compliance with the objectives and procedures of this protocol, or obscure interpretation of the trial data.

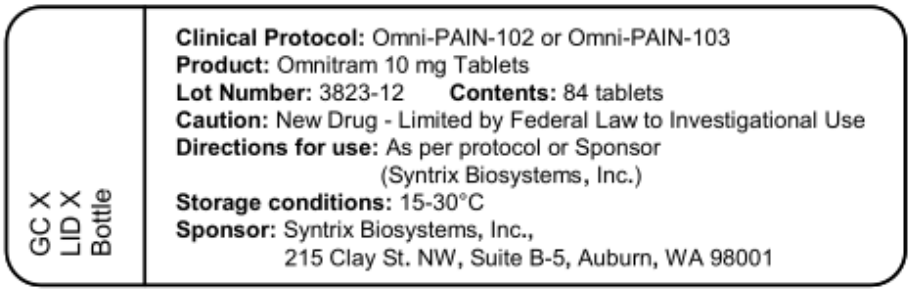
5 STUDY AGENT/INTERVENTIONS

5.1 Study Drug (Desmetramadol)

Desmetramadol is an analgesic in the same class as tramadol (see **FIG. 1** for chemical structures). For comprehensive safety information on the study drug please refer to the Investigator's Brochure.

5.1.1 Formulation, Packaging, and Labeling

Syntrix will provide desmetramadol 10 mg SR tablets. For shipment to the clinical pharmacology unit, desmetramadol tablets will be packaged in bulk in bottles labeled as follows:



5.1.2 Study Drug Storage and Stability

Study drug will be stored at 15-30°C. The supplies should only be accessible to person(s) authorized to dispense them. As soon as the pharmacist or clinical team member receives the study drug, the clinical study supply shipping form must be completed and returned to Syntrix or its designate. The clinical team member should retain a copy of this form for the Investigator's file.

5.1.3 Preparation, Administration, and Dosage of Study Drug

At the time of dosing on Day 1 the subject will be randomized to one of four treatment sequences. Subject treatment randomization will be performed by a pharmacist, investigator, or investigator designee at the clinical pharmacology unit using a treatment randomization list provided by Syntrix Biosystems or designee. The randomization list will: 1) assign a PID#; and 2) indicate the subject's treatment sequence.

The study drug will be administered orally after all protocol specified pre-dose study procedures are completed (see **Appendix A**). Only water is allowed one hour before and one hour after each desmetramadol administration. The study drug will be administered at time 0 hour to each subject in a

sitting posture with approximately 240 mL of water at ambient temperature by trained study personnel in each period. Subjects will be instructed not to chew or crush the tablet(s) but to consume it/them as a whole. Compliance with dosing will be assessed by a thorough check of the oral cavity immediately after dosing. Subjects will remain seated for two hours after dosing in each period except when clinically indicated to change posture or in case of any natural exigency. Thereafter, the subjects will be allowed to engage in normal activities, including eating solid food, but while avoiding severe physical exertion.

5.1.4 Study Drug Shipping and Accountability Procedures

Desmetramadol drug bottles will be shipped protected from moisture and insulated enabling appropriate temperature control with a temperature recording device. The temperature recording device will be returned by the recipient to the shipper or designate for extraction of the shipment temperature data. Syntrix, or its designee, will keep a record of each shipment, along with the temperature data, for future reference.

All used canisters will be retained by the clinical pharmacology unit until the study monitor performs a complete drug accountability audit. Following this accounting, used canisters may be discarded according to clinical pharmacology unit policy, or returned to Syntrix.

5.2 Assessment of Subject Compliance with Study Drug

Assessment of compliance with administration of study drug in this trial is at the level of the Investigator. All study drug will be administered to subjects as inpatients at the clinical pharmacology unit.

5.3 Concomitant Medications and Procedures

Concomitant medication use will be queried from Study Day -1 through Study Day 11, and any reported medications will be recorded on the concomitant medications CRF.

5.4 Precautionary and Prohibited Medications and Procedures

5.4.1 Prohibited Medications and Procedures

- Use of alcohol within 24 hours of day -1 until the end of the study; and grapefruit, grapefruit-related citrus fruits (e.g., Seville oranges, pomelos), or grapefruit juice or grapefruit-related juices within 7 days of study drug administration and until the end of the study.

- MAO Inhibitors (including linezolid), Serotonin-Reuptake Inhibitors, Serotonin-Norepinephrine Reuptake Inhibitors, drugs known to induces or inhibit drug metabolism, including CYP2D6, and other drugs that may affect the serotonergic neurotransmitter systems including, but not limited to, triptans, dextromethorphan, tricyclic antidepressants, bupropion, lithium, tramadol, dietary supplements such as tryptophan and St. John's Wort, and antipsychotics or other dopamine antagonists are prohibited from 14 days before Study Day -1, or 5 half-lives, whichever is longer, until the end of the study (Study Day 11).

- Prescription medications (excluding contraceptive medications or hormone replacement therapy) and nonprescription medications, including dietary supplements and herbal remedies, are prohibited from 14 days before Study Day -1, or 5 half-lives, whichever is longer, until the end of the study (Study Day 11).

- At any time while on study, elective surgical procedures.

6 STUDY PROCEDURES and EVALUATIONS

The information outlined in this Procedures and Evaluations section will be more readily followed if read in conjunction with the tabular listing of this information in the Schedule of Procedures and Evaluations in **Appendix A**. All assessments, unless otherwise specified, should be performed within approximately 15 minutes of the indicated time.

6.1 Screening (≤ 30 days Before Day-1) and Check-In (Day -1)

1. Perform informed consent and obtain signed informed consent form.

2. Assign a Screening Identification number (SID #) and record it in the Screening Enrollment Log.

3. Review contraception requirements with female subjects (**section 4.1**)

4. Perform and record medical history including current medications and those taken within the past 6 weeks, any signs and symptoms being experienced, i.e., adverse events, vital signs, collection of safety lab test specimens, including viral serologies, sample for serum pregnancy test (beta-HCG) if subject is female, urine test for substances of abuse, and ECG. Calculate BMI.

5. Determine if subject meets eligibility criteria. Volunteers who meet all entry criteria can be scheduled for check-in on Day -1.

6. At check-in, assess continued eligibility with respect to the inclusion criteria (including normal vital signs), and exclusion criteria (including oral temperature >38°C or current illness). Obtain interim medical history, perform symptom driven physical examination, and obtain vital signs. Record concomitant medications (including alternative and complementary treatments) and determine if they are within the prohibited drugs (**section 5.4**).
7. Review contraception requirements with female subjects (**section 4.1**)

8. Perform urine test for: substances of abuse; and pregnancy (female subjects only).

6.2 Randomization and Study Drug Treatment Period 1 (Day 1 Through Day 3).

1. Randomization: review Syntrix randomization list to obtain participant identification number (PID#) and treatment sequence. Randomized subject PID# is to be used on all CRFs.

2. On Day 1 determine if subject to be fasted (subject's diet limited to clear liquids for a minimum of 10 hours pre-dose and a minimum of 4 hours after dosing; and diet limited to water only one hour

pre-dose and one hour post-dose), or served a high-fat, high-calorie breakfast approximately to 30 minutes before dosing that is to be consumed within 20 minutes.

3. On Day 1 obtain study drug, provide proper diet (record if fasted or time of high-fat, high-calorie meal consumption), and administer the oral dose according to **Section 5.1.3** and record the time of dosing and the absence of tablets in the oral cavity.

4. Record adverse events and concomitant medications.

5. Collect PK blood and urine (urine only for 30 mg fasted subjects) specimens and process to specimens as indicated in the validated method or PK specimen preparation procedure.

Approximately 35 minutes before the dose obtain PK baseline level blood specimen, and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12, 16, 24, and 32 hours after the dose, obtain a blood specimen as close to the nominal time point for sample collection as possible but in no event not outside the window specified in the **Appendix A Auxiliary**. Collect urine as described in the **Appendix A Auxiliary**. Time of collection of each sample will be recorded in source documents and CRF. The specimens will be stored at the method validated temperature (e.g., -20°C or -80°C) until analysis is performed. If specimens require shipping for analysis, then it will be by overnight courier (split 1 and split 2 must be sent in separate shipments) to the Celerion designated bioanalytical laboratory on dry ice with sufficient dry ice to keep samples at -80°C for 48 hours.

6.3 Study Drug Treatment Period 2 (Day 4 through Day 6)

1. On Day 4 determine if subject to be fasted (subject's diet limited to clear liquids for a minimum of 10 hours pre-dose and a minimum of 4 hours after dosing; and diet limited to water only one hour pre-dose and one hour post-dose), or served a high-fat, high-calorie breakfast approximately 30 minutes before dosing that is to be consumed within 20 minutes.

2. On Day 4 obtain study drug, provide proper diet (record if fasted or times of consumption), and administer the oral dose according to **Section 5.1.3** and record the time of dosing and the absence of tablets in the oral cavity.

3. Record adverse events and concomitant medications.

4. Collect PK blood and urine (urine only for 30 mg fasted subjects) specimens and process to frozen plasma specimens as described in protocol **section 6.2, item #5**.

5. Obtain vital signs.

6.4 Study Drug Treatment Period 3 (Day 7 through Day 9)

1. On Day 7 determine if subject to be fasted (subject's diet limited to clear liquids for a minimum of 10 hours pre-dose and a minimum of 4 hours after dosing; and diet limited to water only one hour pre-dose and one hour post-dose), or served a high-fat, high-calorie breakfast approximately 30 minutes before dosing and that is to be consumed within 20 minutes.

2. On Day 7 obtain study drug, provide proper diet (record if fasted or times of consumption), and administer the oral dose according to **Section 5.1.3** and record the time of dosing and the absence of tablets in the oral cavity.

3. Record adverse events and concomitant medications.

4. Collect PK blood and urine (urine only for 30 mg fasted subjects) specimens and process to frozen plasma specimens as described in protocol **section 6.2, item #5**.

5. Obtain vital signs.

6.5 Study Drug Treatment Period 4 (Day 10 through Day 11)

1. On Day 10 determine if subject to be fasted (subject's diet limited to clear liquids for a minimum of 10 hours pre-dose and a minimum of 4 hours after dosing; and diet limited to water only one hour pre-dose and one hour post-dose), or served a high-fat, high-calorie breakfast approximately 30 minutes before dosing that is to be consumed within 20 minutes.

1. On Day 10 obtain study drug, provide proper diet (record if fasted or times of consumption), and administer the oral dose according to **Section 5.1.3** and record the time of dosing and the absence of tablets in the oral cavity.

2. Record adverse events and concomitant medications.

3. Collect PK blood and urine (urine only for 30 mg fasted subjects) specimens and process to frozen plasma specimens as described in protocol **section 6.2, item #5**.

4. Obtain vital signs.

5. On Day 11, with 24.0 h PK blood collection, collect blood specimen for safety lab tests.

6. Before discharge from the clinical pharmacology unit, review the contraception requirements with female subjects (**section 4.1**).

6.6 Subject Samples

PK samples will be stored frozen for analysis. Safety lab blood collections will be processed by the clinical pharmacology unit laboratory, or designated laboratory.

7 ADVERSE EVENTS

7.1 Specification of Safety Parameters

The safety assessments will be based on clinical AEs reported by the subject or observed by the investigator (or appropriate designee) and laboratory abnormalities considered to be clinically significant by the investigoator (or designee). AEs will be recorded on the AE CRF. Clinical AE relationship to study drug will be determined by the investigator (or appropriate designee) and recorded on the AE CRF. Laboratory AE relationship to study drug will be determined or recorded on the AE CRFs.

7.2 Definitions

7.2.1 Adverse Event

An AE is any untoward medical occurrence in a subject administered the study drug that does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease, whether or not related to the study drug.

7.2.2 Serious Adverse Event

A Serious Adverse Event is defined as an AE occurring while subject is on study resulting in one of the following conditions:

- Death,
- Life Threatening* (defined as a subject at immediate risk of death at the time of the event),
- Inpatient hospitalization or prolongation of existing hospitalization,
- Congenital anomaly or birth defect,
- A persistent or significant disability/incapacity.

*Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a SAE when, based upon medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.2.3 Unexpected Adverse Event

An adverse event is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

7.2.4 Life-Threatening Event

An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

7.3 Assessment of Adverse Events

Assessment of clinical AE/SAE must be by the Investigator or appropriate designee, and should include the intensity (severity) of the event and the relationship to study drug.

7.3.1 Severity Grading

Assessment of an AE/SAE severity must be made by the Investigator, whether clinical or laboratory. Severity of clinical and laboratory adverse events will be assessed by the study clinician using a protocol-

defined grading system as represented in **Appendix C** and **Appendix D**, adapted from similar toxicity grading tables used for healthy adult volunteers in vaccine clinical trials (CBER September 2007).

7.3.2 Definition of Relationship of Adverse Events to Study Drug

All AEs will be recorded, and assessment of their relationship to study drug will be based on the clinical judgment of the study clinician according to the definitions set out below. For every AE, the study clinician will evaluate its relationship to the study drug administration based on the temporal relationship to administrations and the toxicity profile of any concomitant medication. (NOTE: relationship is not a factor in determining whether an AE is or is not reported).

Relationship of AE to study drug:

- ***Definitely related***: The event:
  - follows a reasonable temporal sequence from the time of administration; *and/or*
  - follows a known response pattern to the administration; *and*
  - could not have been produced by other factors such as the subject's clinical state, therapeutic intervention, or concomitant therapy; *and*
  - either occurs immediately following administration, oIr improves on stopping administration.

- ***Probably related***: The event:
  - follows a reasonable temporal sequence from the time of administration; *and/or*
  - follows a known response pattern to the administration; *and*
  - could not have been produced by other factors such as the subject's clinical status, therapeutic intervention, or concomitant therapy.

- ***Possibly related***: The event could have been produced by other factors (such as a clinical illness), but it either:
  - follows a reasonable temporal sequence from the time of administration; or
  - follows a known response pattern to the administration

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, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possible" soon after discovery, it can be flagged as requiring more information and later be upgraded to probable or certain as appropriate.

- ***Not related***: The event is clearly related to other factors such as the subject's clinical state, or other concomitant therapy. The alternative, definitive etiology must be documented.

7.4 Reporting Adverse Events

All AEs, regardless of severity or presumed relationship to study drug, will be documented on the AE CRF. All SAEs, Grade 3 and Grade 4 AEs, must be reported by the Principal Investigator (or designee)

directly to Syntrix Pharmacovigilance within 24 hours by e-mail or telephone. The reporting sequence for all SAEs, Grade 3 AEs, and Grade 4 AEs will be as follows:

1. The Principal Investigator (or designee) must report the event immediately (within 24 hours) to Syntrix Pharmacovigilance.

2. Syntrix Pharmacovigilance will then complete and forward an incident report by e-mail, containing all relevant information about the subject to the Syntrix Medical Monitor (MM) within 48 hours of receipt of the report of the event.

3. The MM must provide a written report by e-mail to Syntrix Pharmacovigilance within 2 working days of receipt of the initial incident report. This report must provide a recommendation as to whether the individual subject may continue to receive study drug (if more drug is scheduled).

4. Syntrix Pharmacovigilance will forward the MM's written report and recommendation by e-mail to the Principal Investigator within 2 working days of receipt.

The Principal Investigator will meet the IRB reporting requirements.

All SAEs, Grade 3 AEs, and Grade 4 AEs judged to be probably or definitely related to study drug will be reported by Syntrix to the FDA within 15 days of receipt of the report of the event.

7.5 Follow-up of Subjects with Adverse Events

All AEs must be followed to resolution or stabilization.

8 Assessment of Risk

8.1 Criteria for Interrupting the Study Based on Dose-Limiting Toxicity

8.1.1 Rules for Discontinuing Study Drug in an Individual Subject

The following are individual stopping rules, as determined by the principal investigator, that would result in a subject being withdrawn from further study drug administration. The expected toxicity profile of desmetramadol, the metabolic product of tramadol, should be very similar to the tramadol toxicity profile, which is well described (see IB).

1. Occurrence of a seizure.

2. Signs or symptoms indicating serotonin syndrome, which may include mental status changes (e.g., agitation, hallucinations, coma), autonomic instability (e.g., tachycardia, labile blood pressure, hyperthermia), neuromuscular aberrations (e.g., hyperreflexia, incoordination) and/or gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea). Serotonin syndrome, in its most severe form can resemble neuroleptic malignant syndrome, which includes hyperthermia, muscle rigidity, autonomic instability with possible rapid fluctuation of vital signs, and mental status changes.

3. Inability to tolerate study drug (e.g., excessive nausea, repeated vomiting, excessive diarrhea, respiratory depression, excessive agitation or anxiety). Respiratory depression is defined as respiratory rate of < 10 breathes/minute.

4. Any clinical significant condition (e.g., a condition for which continued study drug administration may pose additional risk), including but not limited to the following:

    - Pregnancy
    - Any Grade 4 systemic symptom, clinical or laboratory abnormality, or AE regardless of relationship to study drug.

5. Any change in health status during the study period that would affect eligibility status of the subject as defined by all of the study entry criteria.

8.1.2 Suspension of the Study

If safety concerns arise during the study, the Sponsor may suspend, amend or terminate the study. Furthermore, if the study is suspended, the Sponsor may resume the study once the safety concern is resolved.

8.1.3 Confirmatory Testing of Significant (grade 3 and 4) Laboratory Abnormalities

Any laboratory abnormality, obtained after study drug administration, of grade 3 or higher should be repeated before declaring the abnormality legitimate for the purpose of following the algorithms in this section. Grade 1 and grade 2 abnormalities are not subject to repeat testing. To declare the initial grade 3 or higher laboratory abnormality legitimate, confirmatory testing must (1) be completed as soon as possible following the initial abnormality, and (2) exhibit at least some abnormality consistent with the original finding (albeit perhaps not of the same severity). If confirmed in this fashion, the initial finding is declared legitimate and the finding acted upon per protocol. If repeat testing demonstrates a laboratory value completely within normal range, the initial value is declared illegitimate for management purposes and treatment and reporting is continued per protocol as if the first finding was normal.

8.2 Subject Withdrawal from the Study

Subjects will be free to withdraw from the study at any time and for any reason. Any subject who withdraws from the study, regardless of reason, will not be re-enrolled in this study. Reasons for withdrawal will be recorded on the appropriate CRF. The final report will include reasons for withdrawal.

8.3 Replacement of a Subject Who Withdraws from Study Treatment

If subjects withdraw from study before study Day 11, additional subjects may be screened and enrolled at the discretion of the Sponsor (see Section 9.7 Sample Size Considerations). The Syntrix Study Director or designee will provide instructions on replacement treatment assignments.

9 STATISTICAL CONSIDERATIONS

Descriptive statistics for continuous variables will consist of the mean, median, geometric mean (where appropriate), standard deviation, CV, minimum, and maximum values. For categorical variables, the number and percentage of each category will be displayed for each treatment group. An alpha level of 5% will be used. All statistical tests will be two-tailed unless otherwise stated.

Calculations and statistical analyses will be described in further detail as part of a statistical analysis plan (SAP).

9.1 Randomization Procedures

All subjects who meet all entry criteria are eligible to be randomized and receive study drug treatments. On Day 1 subjects are randomized. The PID # is then conveyed to study staff for treatment assignment.

A minimum of 24 subjects will be enrolled and randomized. The randomization list will be provided by Syntrix Biosystems or designee. Randomization of subjects will be balanced across the four treatment sequences in the 4x4 Williams' design. Balanced randomization requires N be evenly divisible by four. The treatment assignments will be made using a computer-generated random list. The randomization list will be provided to the pharmacist or designee responsible for study drug diet and study drug disbursement. This randomization list will match treatment assignment to PID #. As subjects are assigned a PID # on Day1, the pharmacist or designee will link that number to the treatment sequence assigned.

9.2 Plan for Statistical Summaries and Analyses

9.2.1 General Considerations

The safety and intent-to-treat (ITT) populations are defined as all subjects who receive at least one dose of study drug. The per-protocol (PP) population is defined as subjects who meet the inclusion/exclusion criteria, complete Study Day 11, receive all four doses of study drug, and have provided for each study drug dose, at least 13 of the 15 PK blood samples. The evaluable population are those subjects with calculable values for one or more of $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and/or C_{max}.

9.2.2 Disposition of Subjects

The following information will be summarized:

- Number of subjects randomized.
- Number (%) of subjects receiving all four study drug doses.
- Number (%) of subjects who discontinued the study and reasons for discontinuation.
- Number (%) of subjects in the per-protocol population and reasons for those excluded.

- Number (%) of subjects with at least one protocol violation and the protocol violation.
- Additional as may be defined in the SAP.

Protocol violations will be identified by the Syntrix Medical Monitor (or designee) for the trial.

9.2.3 Demographic Characteristics

The summaries and statistical analyses of demographic characteristics will be provided for the safety population as defined in the SAP.

9.2.4 Safety Endpoints

AEs will be coded using the most current version of Medical Dictionary for Regulatory Activities (MedDRA®) available at Celerion and summarized by treatment for the number of subjects reporting the treatment emergent adverse event (TEAE) and the number of TEAEs reported. A by-subject AE data listing including verbatim term, coded term, treatment, severity, and relationship to treatment will be provided.

Safety data including ECGs, vital signs assessments, clinical laboratory results, will be summarized by treatment and point of time of collection.

Descriptive statistics using appropriate summary statistics will be calculated for quantitative safety data as well as for the difference to baseline, when appropriate.

Concomitant medications will be listed by subject and coded using the most current version of the World Health Organization (WHO) drug dictionary available at Celerion. Medical history will be listed by subject.

9.2.4.1 Dropouts Due to AEs

The number (%) of subjects who dropped out due to AEs will be summarized for each treatment group.

9.2.4.2 Laboratory Data

Summaries of the actual value and change from baseline for all laboratory tests will be provided. A tabulated summary of laboratory abnormalities by toxicity grade will also be provided.

9.2.4.3 Vital Signs

Summaries by treatment group for all vital signs will be provided for each treatment.

9.2.4.4 Interim Safety Analyses

There will be no interim safety analyses for this study.

9.2.4.5 Missing Data

Subjects with missing data will be included in the analyses. There will be no imputation of missing values.

9.2.5 PK Analysis

The specified endpoints (PK responses) will be computed from the plasma analyte levels and urine volume and analyte levels (urine only for 30 mg fasted subjects), and summarized for each subject by treatment, and for each treatment cohort.

9.3 Planned Interim Analysis

There will be no interim analysis for this study.

9.4 Procedures for Deviations from Planned Statistical Analyses

The planned statistical analyses for this study will be described in detail in a statistical analysis plan (SAP). Modifications or additions to the analyses of study data described herein will be described in the SAP. Any decisions to deviate from the planned analyses described in the protocol will be documented in the clinical study report and an explanation provided.

9.5 Appropriate Method and Timing for Analyzing Outcome Measures

9.5.1 Methods and Timing for the Primary End-Point

Endpoints. The endpoints are PK responses for all measured blood analytes (i.e., each enantiomer of M1 and M5): C_{max}, T_{max}, $T_{1/2}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and if present, T_{lag}) derived from blood specimens obtained before and after each single dose study drug treatment. The urine endpoints are CL_{M1}, CL_{M5}, CL_{CR}, and the mass of M1 and M5 excreted(urine only for 30 mg fasted subjects). Exploratory urine endpoints include calculating the rate constants for total elimination (k_{el}), renal elimination (k_e), and fraction excreted (f_e) by plotting the rate of excretion ($\Delta U/\Delta t$) vs. the midpoint time of each interval They will be reported for the ITT, evaluable and PP populations. The PK responses relevant to dose-proportionality and food effect are M1 $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max}. Dose-proportionality and food-effect will be established using the evaluable population.

Methods. Dose-proportionality will be established according to the power-model $Y_i = \alpha \cdot D_i^{\beta}$ (Smith 2000), where Y is the pharmacokinetic response (e.g., AUC, C_{max}), α is the coefficient, β the exponent, D the dose, and i the fasted dose levels to be explored (i.e., i = 1, 2 and 3 correspond to the 10, 20 and 30 mg doses, respectively). The power-model is linearized by natural log transforming the equation to:

$$\ln(Y_i) = \alpha + \beta \cdot \ln(D_i) \qquad \text{(Equation 1)}$$

Strict dose linearity yields a slope β equal to 1.

The common acceptance range of 0.8–1.25 for dose proportionality is modified to account for the ratio of dose levels employed in this study as follows: [1+ln(0.8)/ln(3), 1+ln(1.25)/ln(3)], which yields an

acceptance range of [0.7969, 1.2031]. A mixed-effects statistical model based on Equation 1 can be used to account for correlation between repeated measurements in a given subject (Smith 2000). The random effects, as well as the fixed effects (α and β) and their 90% CI, can be estimated with MIXED procedure of SAS and other statistical packages.

If the 90% CI of β lies entirely within [0.7969, 1.2031], dose-proportionality will be said to be proven for the PK response.

If at least one of the limits of the 90% CI of β lies outside [0.7969, 1.2031], dose proportionality will be said to not be proven for the PK response (inconclusive: e.g., higher variability and/or higher deviation from dose-proportionality than expected).

If the 90% CI of β lies entirely outside [0.7969, 1.2031], dosing is disproportionate for the PK response.

Absent food effect will be established if the 90 percent CI for the ratio of population geometric means between fed and fasted 30 mg desmetramadol doses, based on log-transformed data, is contained in the equivalence limits of 0.80-1.25 for $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max} (FDA Guidance for Industry. *Assessing the Effects of Food on Drugs in INDs and NDAs-Clinical Pharmacology Considerations.* (Draft) CDER. February 2019).

The above dose-proportionality and food effect analyses will be carried out using PK responses derived from the sum of (+) and (-) O-desmethyltramadol. Exploratory dose-proportionality and food effect analyses may optionally be carried out for PK responses derived from individual enantiomers.

9.6 Study Hypotheses

Desmetramadol is (1) dose-proportional for the desmetramadol doses of 10 mg, 20 mg and 30 mg, and (2) oral administration of desmetramadol with food does not significantly affect its rate or extent of absorption, and therefore, desmetramadol can be administered without regard to food.

9.7 Sample Size Considerations

Sample Size. A minimum of 24 subjects will be randomized. Desmetramadol and tramadol are closely related chemicals and are therefore expected to have a similar intra-patient coefficient of variation and food-effect. Published studies indicate a ~15% intra-subject coefficient of variation for immediate release tramadol (Najib 2009, ESP_Pharma_Limited 2010, Dhanure 2013), and the Ultram label indicates that "oral administration of Ultram with food does not significantly affect its rate or extent of absorption, therefore, Ultram can be administered without regard to food."

	Intra-Subject CV		
	Najib 2009 (N=24)	ESP Pharma Limited 2010 (N=18)	Dhanure 2013 (N=35)
$AUC_{(0\text{-}t)}$	12%	12%	5.6
$AUC_{(inf)}$	12%	11%	6.3
C_{max}	12%	15%	15.0

Using a conservative estimate of 20% for the intra-subject coefficient of variation (CV) of desmetramadol, and a 5% level of significance, a sample size of 24 subjects is considered sufficient to provide 89.6% power (essentially 90% power) to establish dose-proportionality and absent food effect, assuming no multiplicity and a fed/fasting ratio of 0.95 and a 5% deviation from 1 for the slope β in dose proportionality. Based on a study drop-out rate of ~30% in the Ultram label, up to 8 additional subjects (alternates) might be randomized to enable a per protocol population of 24 subjects with blood draws at each treatment period (based on our completed phase 1 study in 43 subjects, dropouts are unlikely to exceed 5%; see **Section 1.2.2**).

Sensitivity Analysis. ICH E9, "It is important to investigate the sensitivity of the sample size estimate to a variety of deviations from these assumptions and this may be facilitated by providing a range of sample sizes appropriate for a reasonable range of deviations from assumptions." Generally power is set to at least 80% in BE studies, and a minimum of 12 evaluable subjects should be included in any BE study (FDA 2001). However, even with a power of 80%, 1 in 5 studies will fail by mere chance alone, and thus higher power is preferable. Based on the assumptions above of a 5% deviation from 1 for the slope β in dose proportionality and a 20% intra-subject CV, 20 subjects provide at least 80% power and 24 subjects provide nearly 90% power (table below).

	N*								
	12	14	16	18	20	22	24	26	28
5% β deviation									
CV									
15%	80.0%		90+%						
20%			73.5%	79.1%	83.5%		89.6%	90+%	
25%								78.0%	80.0%
10% β deviation									
CV									
15%					80+%				
20%							70.0%		<80%
25%									<<80%

*Note that a balanced four-sequence Williams' design requires an N evenly divisible by 4.

If the intra-subject CV is actually closer to the published value for tramadol of 15%, then as few as 12 subjects provide at least 80% power and 16 subjects provide at least 90% power (see table). On the other hand, if the intra-subject CV is much worse at 25%, then the planned enrollment of 24 subjects fails to provide the minimum threshold of 80% power. Likewise, if the deviation from 1 in the slope β is 10% instead of 5%, then only a 15% intra-subject CV and 20 enrolled subjects will provide the minimum desired power of 80%. Higher intra-subject CVs of 20% and 25% both fail to provide at least 80% power with this degree of deviation in β, even with as many as 28 subjects. Based on this sensitivity analysis and the small (6-15%) actual intra-subject CV for the closely related molecule tramadol, the planned enrollment of 24 subjects provides a comfortable margin of power should the already conservative estimates of 20% intra-subject CV and 5% β deviation prove correct or even slightly larger.

9.8 Maintenance of Trial Randomization List

Syntrix and the site pharmacist, investigator or investigator designee will maintain the randomization list.

9.9 Subject Enrollment and Follow-Up

The total number of subjects who complete the PK analysis in all 4 Periods is targeted to be 24. The total duration of accrual is 4 months, and duration from first patient enrolled until final subject contact is about 6 weeks.

9.10 Safety Review

No formal interim statistical analyses will be performed for safety.

9.11 Final Analysis Plan

A formal statistical analysis plan will be developed and finalized prior to data analysis.

10 ADMINISTRATIVE PROCEDURES

10.1 Institutional Review Board Approval

The protocol for this study has been designed in accordance with Good Clinical Practice and ICH E6. The review of this protocol by the Institutional Review Board (IRB) and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles codified in Title 21 Code of Federal Regulations (CFR) Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing and submitting documents to the relevant IRB, and obtaining written IRB approval for this study. The approval will be obtained prior to study initiation.

10.2 Informed Consent

A study informed consent will be obtained from each subject. The Investigator or designee will explain the nature of the investigation and the risks involved to each subject prior to enrollment, and will obtain written informed consent. The subject will also be informed that he/she is free to voluntarily withdraw from the study at any time.

10.3 Direct Access to Source Data/Documents, Study Monitoring, Data Collection

The Investigator will allow representatives of Syntrix (or their designee) to periodically monitor, at mutually convenient times during and after the study, all CRFs and corresponding source documents for each subject. It is important that the Investigator or staff is available at these visits. The monitoring visits provide Syntrix with the opportunity to evaluate the progress of the study, to verify the accuracy and completeness of CRFs, to resolve any inconsistencies in the study records, as well as assuring that all protocol requirements, applicable regulations, and Investigator's obligations are being fulfilled. The Investigator must maintain all source documents, i.e., all information, original records of clinical findings, observations, or other activities necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to clinical charts, laboratory notes, pharmacy dispensing records, and subject files and records kept at the pharmacy, at the laboratories, and other medico-technical departments involved in the clinical trial), for possible review. The investigational site will provide direct access to all trial-related source documents, other documents, and reports for the purpose of monitoring and auditing by Syntrix and regulatory authorities.

The Syntrix representative will record the date of each monitoring visit together with a summary of the status and progress of the study. Proposed actions will be confirmed with the Investigator in writing. Telephone contact will be made as necessary during the data collection period and during the data and report writing periods.

Celerion standard CRFs will be supplied. CRFs are produced, stored electronically, and are available to the designated study team members. Each CRF is reviewed and signed by the PI. The final signed CRFs are provided to the Sponsor in the format as decided upon between Celerion and the Sponsor (e.g., CD, flashdrive, SFTP). This will be documented in the DMP (if applicable).

If CRF entries are modified, the initials of the associate making the change as well as the date and reason for the change will be captured in an electronic audit trail. The clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol and the applicable regulatory requirements.

Standard operating procedures are available for all activities performed at Celerion relevant to the quality of this study. Designated personnel of Celerion will be responsible for implementing and maintaining quality assurance (QA) and quality control systems to ensure that the study is conducted, and that data are generated, documented and reported in compliance with the study protocol, and GCP requirements as well as applicable regulatory requirements and local laws, rules and regulations relating to the conduct of the clinical study.

All clinical data will undergo a 100% quality control check prior to clinical database lock. Edit checks are then performed for appropriate databases as a validation routine using SAS® or comparable statistical program to check for missing data, data inconsistencies, data ranges, etc. Corrections are made prior to database lock.

The Clinical Study Report will be audited by the QA department and the QA audit certificate will be included in the study report.

10.4 DATA ANALYSIS

Data will be handled and processed according to Celerion SOPs, which are written based on the principles of GCP. A brief description of the statistical analysis is included below, detailed methodology for all summary and statistical analyses of the data collected in this trial will be documented in a statistical analysis plan (SAP) prepared by Celerion and agreed upon by the Sponsor. The SAP may modify the plans outlined in the protocol; however, any major modifications of the primary endpoints and/or their analysis will also be reflected in a protocol amendment. If deemed appropriate, additional statistical analyses other than those described in this section may be performed and included in the plan.

10.5 Modification of the Protocol

No deviations from the protocol are permitted. Syntrix may approve minor exceptions on a case-by-case basis. If modification of the protocol is necessary, the modification must be initiated and confirmed in writing by Syntrix, and the Investigator will inform the IRB and not institute the modification until approved by the IRB.

10.6 Protocol Deviation(s)

A protocol deviation is any noncompliance with the clinical trial protocol or Good Clinical Practice. Syntrix's Medical Director should be consulted if there is uncertainty as to whether a protocol deviation has occurred. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. Protocol deviations will be recorded by the site and included in the final study report. It is the responsibility of the site to use continuous vigilance to identify and report deviations. Protocol deviations must be sent to the IRB per their guidelines.

10.7 Departure from the Protocol

When an emergency involving an individual occurs a departure from the protocol may be required. This protocol departure will be limited to this individual emergency. The Investigator, or other physicians in attendance, in such an emergency will, if circumstances and time permit, contact Syntrix immediately by telephone. The protocol deviation forms will completely describe the departure from the protocol and state the reasons for such departure. The IRB must be notified immediately if the departure from the protocol affects the safety or rights of the subject.

10.8 Suspension of the Study

If safety concerns arise during the study, the Sponsor may suspend, amend or terminate the study. Furthermore, if the study is suspended, the Sponsor may resume the study once the safety concern is resolved.

10.9 Study Termination by the Sponsor

Syntrix retains the right to terminate the study for any cause, suspending subject enrollment and removing all investigational products and related study materials from the study site at any time. Specific instances, which may precipitate such termination at a site, are as follows:

- Deviation from protocol requirements.
- Inaccurate or incomplete data recording on a recurrent basis.
- Unauthorized use of investigational products.
- Delinquent fulfillment of obligation on the part of the Investigator with regard to adverse reaction reporting, unacceptable subject enrollment, or other responsibilities as outlined in this protocol.

10.10 Use of Information and Publications

Publication of the results of this study is encouraged subsequent to full data analysis. No part of the results of the study, or any of the information provided by the Sponsor to the Investigator for the purposes of performing the study, will be published, or passed on to a third party, without prior review by the Sponsor. The Investigator, or anyone else working on the study, will submit all proposed publications, papers, abstracts or other written materials related to the Study, or an outline of any proposed oral presentation with respect thereto, to the Sponsor at least one month prior to (i) submission of such written materials for publication, or (ii) any proposed oral disclosure to a third party.

The Sponsor shall have the right to comment on such written material or outline; such comments shall be considered in good faith by the Investigator in determining the final form of disclosure. In the event patentable material is identified in the data, the Sponsor may delay publication for up to six months to submit the appropriate patent applications. Notwithstanding any of the above, the Investigator or anyone else working on the Study may not include any confidential information in any such publication or disclosure.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase 1 trials), would be exempt from this policy. Syntrix will register this trial in a public trials registry.

The Investigator is obliged to provide the Sponsor with complete test results and all data derived from the study. Only the Sponsor may make information obtained during and from the study available to regulatory agencies, except as required by regulation.

10.11 Record Retention

The Investigator or Investigative site must retain the clinical study until a minimum of two years following the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least two years have elapsed since the formal discontinuation of clinical development of the study agent. Syntrix Biosystems will notify sites when records can be destroyed. In the event this information is not communicated to the sites, permission must be obtained from Syntrix Biosystems before records can be destroyed.

10.12 Exclusion of Minorities, and Children (Special Populations)

Children and subjects less than 19 years of age are excluded from this phase 1 study. Thus, the study will not enroll children, prisoners, or other vulnerable populations. The study is therefore exempt from 45 CFR 46 Subparts B, C and D.

Minorities and underinsured persons are encouraged to participate. Since the sponsor pays for the study medication, lab tests and examination costs, there are no impediments to anyone's participation in the study.

10.13 Subject Confidentiality

Subject confidentiality will be maintained, and neither full names nor social security numbers will be entered into the database. Subject confidentiality will be strictly held in trust by the participating Investigators and their staff, and the Sponsor and their representatives.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the Sponsor.

Appendix A: Schedule of Procedures/Evaluations

Procedure	Screen Days -31 to -1	Day Of Study -1	1	2	3	4	5	6	7	8	9	10	11[7,8]
In-CPU Stay		X	X	X	X	X	X	X	X	X	X	X	X
Randomization			X										
Study Drug Administration			X			X			X			X	
Clinical													
Informed Consent	X												
Review Contraception Requirements with female subjects	X	X											X
Medical History	X	X[1]											
Physical Exam include height, weight, BMI calculation.	X	X[1]											
Vital signs[2]	X	X	X			X			X			X	X
ECG	X												
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events			X	X	X	X	X	X	X	X	X	X	X
Safety Lab Tests													
CBC with Differential[3]	X												X
BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, albumin, total protein, sodium, potassium, chloride, bicarbonate, calcium, glucose (random),	X												X
PT, PTT	X												
HIV, Hepatitis B & C Serology	X												
UA[3]	X												
Urine Test for Substances of Abuse[4]	X	X											
Pregnancy Test[5]	X	X											
Samples for Pharmacokinetic Analysis[6]			X	X		X	X		X	X		X	X
Discharge													X

[1] Interim history and symptom drivenphysical examinations. Do not include height or BMI calculation.

[2] Vital signs (blood pressure, pulse, respiratory rate, and temperature) will be obtained in the sitting position at screening, on clinical pharmacology unit admission day, before each study drug dose, 30-60 minutes after study drug administration, and before discharge. If the initial RR assessment indicates <10/min, then the RR assessment will be repeated with the rate determeined over 60 seconds.

[3] Complete blood count includes: WBCs, platelets, RBCs, hemoglobin, hematocrit. Differential includes % and absolute number of: neutrophils (all phases of development), lymphocytes (typical and atypical), monocytes, eosinophils, and basophils. Urinalysis includes: appearance, and by dipstick pH, specific gravity, protein, sugar (glucose), keytones, bilirubin, leukocyte esterase or nitrites, blood, urobilinogen. If on the dipstick protein, leukocyte esterase or nitrites, or blood is positive, then a microscopic analysis of the urine will be performed.

[4] Substances to be tested inclue: amphetamines, barbituates, benzodiazepines, opiates, cocaine, cannabinoids, and ethanol.

[5] Female subjects only. At screening obtain a serum pregnancy test. At check-in (Day -1) obtain a urine pregnancy test.

[6] See **Appendix A Auxilliary** that follows this page for collection details.

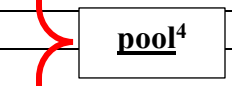
[7] On day 11 safety lab blood collections may be done at time of 24 hour PK blood specimen collection; other procedures should be done around the time of the final PK blood specimen collection prior to discharge.

[8] If a subject terminates early, then Day 11 procedures, as noted in Appendix A, should be completed before subject terminates from the study.

Appendix A Auxiliary: Detailed Pharmacokinetic Sampling Schedule

Days 1-2, 4-5, 7-8 and 10-11 (urine only for 30 mg fasted subjects).

Blood collection[1] (+)-M1, (-)-M1, (+)-M5, (-)-M5	**Urine collection**[2] M1 and M5 achiral, 24 hr creatinine	**Blood collection**[3] Creatinine for 24 hr CL_{CR}
pre-dose	collect pre-dose void (negative control)	
0.5 hr (+/- 5 minutes)		
1.0 hr (+/- 10 minutes)		
1.5 hr (+/- 10 minutes)		
2.0 hr (+/- 10 minutes)	0.0 to 2.0 hour interval	
2.5 hr (+/- 10 minutes)		
3.0 hr (+/- 10 minutes)		
3.5 hr (+/- 10 minutes)		
4.0 hr (+/- 15 minutes)	2.0 to 4.0 hour interval	
6.0 hr (+/- 15 minutes)	4.0 to 6.0 hour interval	
8.0 hr (+/- 15 minutes)	6.0 to 8.0 hour interval	
12.0 hr (+/- 15 minutes)	8.0 to 12.0 hour interval	12.0 hr (+/- 15 minutes)
16.0 hr (+/- 15 minutes)	12.0 to 16.0 hour interval	
24.0 hr (+/- 15 minutes)	16.0 to 24.0 hour interval	
32.0 hr (+/- 15 minutes)	24.0 to 36.0 hour interval	



Footnotes:

1. Collect in K2 EDTA tubes. Centrifuge, split and freeze two duplicate 1 mL aliquots for each time point.
2. Record urine volume collected in each interval. Freeze two duplicate 1 mL aliquots from each interval.
3. Collect blood at 12 hours (mid-point of 24 hour urine collection) in order to compute 24 hour creatinine clearance.
4. After all urine aliquots are obtained from the intervals in the first 24 hours, pool urine collected between 0.0 and 24.0 hours and mix. Take two duplicate 1 mL aliquots and use one to quantitate urine creatinine, and freeze the other one as a backup. Creatinine clearance (CL_{CR}) may be computed with the urine level of creatinine in 24 hours of urine and the blood level of creatinine measured at the time midpoint (i.e., at 12 hours).[1] The clearance mechanism of M1 and M5 is less likely to involve only glomerular filtration and no renal tubular secretion or absorption if CL_{M1} and CL_{M5} resemble CL_{CR} across doses.

[1] One online calculator is at:
http://www-users.med.cornell.edu/~spon/picu/calc/crclcalc.htm

Appendix B. Calculation of Selected Inclusion Criteria

1. Inclusion Criteria: GFR Estimate by Cockcroft-Gault Formula

$$GFR = \frac{[(140 - age) \times weight]}{72 \times SCR}$$

where, age is in years
weight is in kg
serum creatinine (S_{CR}) is in mg/dL.
GFR is ml/min

in females, the result is multiplied by 0.85 (Cockcroft 1976). The Cockcroft-Gault value is not adjusted for body surface area (BSA), and in this protocol must be equal to or greater than 60 ml/min. An online Cockcroft-Gault calculator is available at: http://nephron.com/cgi-bin/CGSI.cgi .

2. Inclusion Criteria: Body Mass Index (BMI)

BMI = (Weight in Pounds x 703)/(Square of Height in Inches)

Examples:

Weight = 188 lbs	Weight = 258 lbs
Height = 72 inches (6 feet)	Height = 72 inches (6 feet)
BMI = 25.5	BMI = 35.0

Online BMI calculators are also available (e.g. http://www.nhlbisupport.com/bmi/bmicalc.htm).

Clinically accepted guidelines note that a BMI from 18.5-24.9 is normal; 25.0 to 29.9 is overweight; a BMI of greater than or equal to 30.0 is obese; and a BMI of greater than or equal to 35.0 is morbidly obese.

Appendix C: Toxicity Grading Scale for Clinical Laboratory Values[1]

Parameter	Normal Range (Grade 0)	Grade for Abnormal Results (Value or Change from Reference) [1]			
	Normal Values	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
BUN (mg/dL)		23-26	27-31	>31	Requires dialysis
Creatinine (mg/dL)		> ULN - 1.7	1.8 - 2.0	2.1 - 2.5	> 2.5 or requires dialysis
ALT (SGPT) (U/L)		> ULN - 2.5 x ULN	> 2.6 - 5.0 x ULN	> 5.1 - 10 x ULN	> 10 x ULN
AST (SGOT) (U/L)		> ULN - 2.5 x ULN	2.6 - 5.0 x ULN	5.1 - 10 x ULN	> 10 x ULN
PT (prothrombin time)		>ULN – 1.2 x ULN	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT (partial tthromboplastin time)		>ULN – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 -1.5 x ULN	> 1.5 x ULN
WBC (K/µL or 10^9/L)		> ULN - 15.0 2.5 - < LLN	> 15.0 - 20.0 ≥ 1.5 - < 2.5	> 20.0 - 25.0 ≥ 1.0 - < 1.5	> 25.0 < 1.0
Hemoglobin (g/dL)		M: 12.5 - < LLN F: 11 - < LLN	M: 10.5 - 12.4 F: 9.5 - 10.9	M: 8.5 - 10.4 F: 8.0 - 9.4	< 8.5 < 8.0
Platelet count (K/µL or 10^9/L)		100 - < LLN	50 - 99	< 50	

[1] = FDA Guidance for Industry Toxicity Grading Scale for Healthy Adults Enrolled in Preventive Vaccine Clinical Trials, 2007. ULN=upper limit of normal; LLN=lower limit of normal.

Appendix D: Toxicity Grading Scale for Clinical AEs[1]

VITAL SIGNS				
	Grade 1 Mild	**Grade 2 Moderate**	**Grade 3 Severe**	**Grade 4 Potentially Life-threatening**
Fever: oral (˚C) (˚F)	38.0 - 38.4 100.4 - 101.1	38.5 - 38.9 101.2 - 102.1	39.0 - 40.0 102.2 - 104	> 40.0 > 104
Tachycardia - bpm	101 - 115	116 - 130	>130	ER visit or hospitalization for arrhythmia
Hypertension (systolic) mm Hg	141 - 150	151 - 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) mm Hg	91 – 95	96 - 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) mm Hg	85 – 89	80 - 84	<80	ER visit or hospitalization for hypotensive shock
SYSTEMIC (GENERAL)				
Nausea/Vomiting	No interference with activity or 1 - 2 episode in 24 hours	Some interference with activity or >2 episodes in 24 hours	Prevents daily activity or requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools in 24 hours	4-5 loose stools in 24 hours	> 5 watery stools in 24 hours or outpatient IV hydration	Requiring significant/urgent medical care or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; use of narcotic pain reliever or prevents daily activity	Requiring significant/urgent medical care or hospitalization
Malaise/fatigue, muscle pain, chills, anorexia (loss of appetite), self-reported symptoms of 'fever' (no temperature taken)	No interference with activity	Some interference with activity	Significant; prevents daily activity	Requiring significant/urgent medical care or hospitalization
Allergic reaction	Pruritus without rash	Localized urticaria (rash)	Generalized urticaria; angioedema	Requiring significant/urgent medical care or hospitalization
Hives (urticaria)	Urticarial lesions covering <10% BSA; topical intervention indicated	Urticarial lesions covering 10 -30% BSA; oral intervention indicated	Urticarial lesions covering >30% BSA; IV intervention indicated	
Illness or clinical adverse event	No interference with activity.	Some interference with activity but does not require medical intervention	Prevents daily activity and requires medical intervention	Requiring significant/urgent medical care or hospitalization

[1] = FDA Guidance for Industry Toxicity Grading Scale for Healthy Adults Enrolled in Preventive Vaccine Clinical Trials, 2007.

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