CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208271
Supporting document/s:	0000
Applicant's letter date:	June 19, 2015
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Product:	Methylnaltrexone Bromide Tablets, 150 mg
Indication:	Treatment of opioid-induced constipation (OIC) in adult patients with chronic non-cancer pain
Applicant:	Salix Pharmaceuticals, Inc.
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer:	Sushanta Chakder, PhD
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1 Executive Summary

1.1 Introduction

Methylnaltrexone bromide (MNTX, MOA-728) is a peripherally active selective mu-opioid receptor antagonist with minimal penetration into the brain at clinically relevant doses. Subcutaneous injections of MNTX (Relistor) is currently approved for the treatment of adult patients with advanced illness with opioid-induced constipation (OIC), who are receiving palliative care, and for the treatment of OIC in adult patients with non-cancer pain. In the current NDA, the Applicant is seeking approval of methylnaltrexone bromide oral tablets (150 mg) for the treatment of OIC in adult patients with chronic non-cancer pain.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies, including pharmacology, safety pharmacology, pharmacokinetics, metabolism and toxicology (general toxicology, genetic toxicology, carcinogenicity and reproductive toxicity) studies were submitted and reviewed under NDA 21964 for the approval of subcutaneous Relistor (Reviewed by Dr. Tamal Chakraborti on March 16, 2012). Since, the approval of SC Relistor, several nonclinical studies were conducted, and the studies were submitted in the current NDA. The studies submitted include a pharmacodynamic study in which the binding of methylnaltrexone and its metabolites, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone on human μ-, κ- and δ-Opioid receptor was assessed *in vitro*. Methylnaltrexone and its metabolites were found to bind with μ- receptors with higher affinities (IC_{50} values, 34 nM to 10.7 μM). A new secondary pharmacology study examined the effects methylnaltrexone, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone on the hERG channels expressed in human embryonic kidney (HEK293) cells. Methylnaltrexone and its metabolites had no significant effect on hERG currents at concentrations up to 100 μM. In an *in vitro* plasma protein binding assay in human plasma, the binding of methylnaltrexone sulfate, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone was low (17% to 41%). Following incubation with human liver microsomes, glucuronidation of methylnaltrexone was lower than that observed in rat and dog microsomes. Methylnaltrexone was not found to undergo CYP450-mediated metabolism *in vitro*; however, it was found to be a competitive inhibitor of CYP2D6.

No new toxicology studies of methylnaltrexone were submitted in the current NDA. Toxicology studies submitted in the initial NDA 21964 and sNDA 21964 were reviewed earlier (for full reviews of the studies, refer to nonclinical review of NDA 21964, dated March 16, 2012). Repeat-dose toxicity studies by the oral route was conducted in mice (up to 90 days), in rats (up to six months), and in dogs (up to 9 months). In addition, the full battery of genotoxicity and reproductive and developmental toxicity studies were conducted with methylnaltrexone bromide, and were reviewed under NDA 21964. Two year carcinogenicity studies in rats and mice were also reviewed under earlier submissions.

In a six-month repeat-dose oral toxicity study in rats, mortality was observed at 500 mg/kg and higher doses, and the NOAEL was considered to be 100 mg/kg/day. At the 100 mg/kg

dose, the mean total systemic exposures (AUC_{0-24}) to MNTX were 552 ng.h/mL in males and 667 ng.h/mL in females. At ≥300 mg/kg/day, globule leukocyte/eosinophilic inflammation in the intestine, increased cellularity in the mesenteric lymph nodes, and epithelial degeneration and edema in the squamous mucosa of the stomach were noted microscopically. The 100 mg/kg NOAEL dose in rats is about 13 times the clinical dose of 450 mg/day.
In a 9-month repeated dose oral toxicity study in dogs, the animals were dosed up to 180 mg/kg/day at the beginning of the study, with the high dose increased to 225 mg/kg/day, then to 250 mg/kg/day by the end of the study. Two female dogs died of apparent gavage errors. No treatment related effects on blood pressures, HR or ECG parameters were observed at any doses. The 60 mg/kg/day dose was considered the NOAEL, and this dose is approximately 8 times the proposed clinical dose of 450 mg/day. Thus, the oral NOAEL doses from the 6-month rat and 9-month dog toxicity studies provide adequate safety margins for the proposed clinical dose, and do not raise any safety concerns.

Methylnaltrexone bromide was negative in a battery of genotoxicity studies. In two year carcinogenicity studies in mice and rats, oral administration of methylnaltrexone bromide at doses up to 400 mg/kg and 300 mg/kg, respectively, did not produce any neoplasms.

Methylnaltrexone bromide at subcutaneous doses up to 150 mg/kg/day had no adverse effect on fertility and reproductive performance of male and female rats.
Methylnaltrexone bromide had no adverse effects on embryofetal development in rats and rabbits at intravenous doses up to 25 mg/kg and 16 mg/kg, respectively.
Methylnaltrexone bromide had no adverse effect on pre- and postnatal development at subcutaneous doses up to 100 mg/kg/day.

1.3.1 Approvability

No nonclinical approvability issues have been identified for the proposed doses of methylnaltrexone bromide tablets.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Applicant's submitted version:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

(b) (4)

Recommended Version:

Risk Summary

The limited available data with RELISTOR in pregnant women are not sufficient to inform a drug-associated risk for major birth defects and miscarriages. In animal reproduction studies, no effects on embryo-fetal development were observed with the administration of intravenous methylnaltrexone bromide during organogenesis in rats and rabbits at doses up to 20 times and 26 times, respectively, the subcutaneous maximum recommended human dose (MRHD) of 12 mg RELISTOR injection per day. The intravenous doses in rats and rabbits are about 0.5 times and 0.7 times, respectively, the oral MRHD of 450 mg/day *[see Data].* Advise pregnant women of the potential risk to a fetus.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

Animal Data

Reproduction studies have been performed with methylnaltrexone bromide administered during the period of organogenesis to rats at intravenous doses up to 25 mg/kg/day (about 20 times the subcutaneous MRHD of 12 mg per day based on body surface area), and did not cause any adverse effects on embryofetal development. In rabbits, intravenous doses of methylnaltrexone bromide up to 16 mg/kg/day (about 26 times the subcutaneous MRHD of 12 mg per based on body surface area) did not show any embryofetal toxicity. The intravenous doses in rats (25 mg/kg/day) and rabbits (16 mg/kg/day) are about 0.5 and 0.7 times, respectively, the oral MRHD of 450 mg/day. A

pre- and postnatal development study in rats showed no evidence of any adverse effect on pre- and postnatal development at subcutaneous doses of methylnaltrexone bromide up to 100 mg/kg/day (about 81 times the subcutaneous MRHD of 12 mg per day, based on body surface area; about 2.2 times the oral MRHD of 450 mg per day).

8.2 Lactation

Proposed Version:

(b) (4)

Recommended Version:

Risk Summary

There is no information regarding the presence of methylnaltrexone in human milk, the effects on the breastfed infant, or the effects on milk production. Methylnaltrexone is present in rat milk *[see Data].* Because of the potential for serious adverse reactions, including opioid withdrawal, in breastfed infants, advise women that breastfeeding is not recommended during treatment with RELISTOR.

Data

Radioactivity appeared in rat milk within 30 minutes of subcutaneous administration of radiolabeled methylnaltrexone bromide and was concentrated up to 24-fold at 8 hours after administration relative to plasma concentrations.

8.4 Pediatric Use

Proposed Version:

(b) (4)

Recommended Version:

Juvenile Toxicity

In juvenile rats administered intravenous methylnaltrexone bromide for 13 weeks, adverse clinical signs such as convulsions, tremors and labored breathing were observed, and the juvenile rats were found to be more sensitive to the adverse effects of methylnaltrexone when compared to adult animals. Juvenile dogs administered intravenous methylnaltrexone bromide for 13 weeks had a toxicity profile similar to adult dogs *[see Nonclinical Toxicology (13.2)]*.

13 NONCLINICAL TOXICOLOGY

Proposed Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Recommended Version:

Carcinogenesis

Two-year oral carcinogenicity studies have been conducted with methylnaltrexone bromide in CD-1 mice at doses up to 200 mg/kg/day (about 81 times the subcutaneous maximum recommended human dose (MRHD) of (b) (4) mg/kg/day based on body surface area) in males and 400 mg/kg/day (about 162 times the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area) in females and in Sprague Dawley rats at oral

doses up to 300 mg/kg/day (about 243 times the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area). The 200 mg/kg/day and 400 mg/kg/day doses in male and female mice are about 2.2 and 4.4 times, respectively, the oral MRHD of 450 mg/day, and the 300 mg/kg/day dose in rats is about 6.5 times the oral MRHD of 450 mg/day, based on body surface area. Oral administration of methylnaltrexone bromide for 104 weeks did not produce tumors in mice and rats.

Mutagenesis

Methylnaltrexone bromide was negative in the Ames test, chromosome aberration tests in Chinese hamster ovary cells and human lymphocytes, in the mouse lymphoma cell forward mutation tests and in the *in vivo* mouse micronucleus test.

Impairment of Fertility

Methylnaltrexone bromide at subcutaneous doses up to 150 mg/kg/day (about 122 times the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area; about 3.3 times the oral MRHD of 450 mg/day) was found to have no adverse effect on fertility and reproductive performance of male and female rats.

13.2 Animal Toxicology and/or Pharmacology

Proposed Version:

(b) (4)

Recommended Version:

13.2 Animal Toxicology and/or Pharmacology

In an *in vitro* human cardiac potassium ion channel (hERG) assay, methylnaltrexone caused concentration-dependent inhibition of hERG current (1%, 12%, 13% and 40% inhibition at 30, 100, 300 and 1000 µM concentrations, respectively). Methylnaltrexone had a hERG IC_{50} of >1000 µM. In isolated dog Purkinje fibers, methylnaltrexone caused prolongations in action potential duration (APD). The highest tested concentration (10 µM) in the dog Purkinje fiber study was about 18 and 37 times the C_{max} at human subcutaneous (SC) doses of 0.3 and 0.15 mg/kg, respectively. In isolated rabbit Purkinje fibers, methylnaltrexone (up to 100 µM) did not have an effect on APD, compared to vehicle control. The highest methylnaltrexone concentration (100 µM) tested was about 186 and 373 times the human C_{max} at SC doses of 0.3 and 0.15 mg/kg, respectively. In anesthetized dogs, methylnaltrexone bromide caused decreases in blood pressure, heart rate, cardiac output, left ventricular pressure, left ventricular end diastolic pressure, and +dP/dt at ≥ 1 mg/kg. In conscious dogs, methylnaltrexone bromide caused a dose-related increase in QTc interval. After a single intravenous dosage of 20 mg/kg to beagle dogs, predicted C_{max} and AUC values were approximately 482 and 144 times, respectively, the exposure at human SC dose of 0.15 mg/kg and 241 times and 66 times, respectively, the exposure at a human SC dose of 0.3 mg/kg. In conscious guinea pigs, methylnaltrexone bromide caused mild prolongation of QTc (4% over baseline) at 20 mg/kg, intravenous. A thorough QTc assessment was conducted in humans [*see Clinical Pharmacology (12.2)*].

In juvenile rats administered intravenous methylnaltrexone bromide for 13 weeks, adverse clinical signs such as convulsions, tremors and labored breathing occurred at dosages of 3 and 10 mg/kg/day (about 2.4 and 8 times, respectively, the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area; about 0.06 and 0.22 times, respectively, the oral MRHD of 450 mg/day). Similar adverse clinical signs were seen in adult rats at 20 mg/kg/day (about 16 times the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area; about 0.43 times the oral MRHD of 450 mg/day). Juvenile rats were found to be more sensitive to the toxicity of methylnaltrexone bromide when compared to adults. The no observed adverse effect levels (NOAELs) in juvenile and adult rats were 1 and 5 mg/kg/day, respectively (about 0.8 and 4 times respectively, the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area; about 0.02 and 0.08 times, respectively, the oral MRHD of 450 mg/day).

Juvenile dogs administered intravenous methylnaltrexone bromide for 13 weeks had a toxicity profile similar to adult dogs. Following intravenous administration of methylnaltrexone bromide for 13 weeks, decreased heart rate (13.2% reduction compared to pre-dose) in juvenile dogs and prolonged QTc interval in juvenile (9.6% compared to control) and adult (up to 15% compared to control) dogs occurred at 20 mg/kg/day (about 54 times the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area; about (b) (4) times the oral MRHD of 450 mg/day). Clinical signs consistent with effects on the CNS (including tremors and decreased activity) occurred in both juvenile and adult dogs. The NOAELs in juvenile and adult dogs were 5 mg/kg/day (about 14 times the subcutaneous MRHD of (b) (4) mg/kg/day based on body surface area; about (b) (4) times the oral MRHD of 450 mg/day).

2 Drug Information

2.1 Drug

CAS Registry Number: 73232-52-7

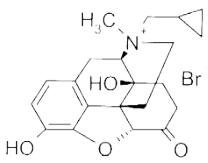
Generic Name: N-methylnaltrexone bromide

Code Name: MOA-728

Chemical Name: N-(cyclopropylmethyl)-noroxymorphone methobromide

Molecular Formula/Molecular Weight: $C_{21}H_{26}NO_4$. Br /436

Structure or Biochemical Description:



Pharmacologic Class: Opioid receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

1. IND 64,583 (Methylnaltrexone Bromide Injection, Progenics Pharmaceuticals, Inc.)
2. IND 67,452 (Methylnaltrexone Bromide Tablet, Wyeth Pharmaceuticals, Inc.)
3. NDA 21-964 (Methylnaltrexone Bromide Injection, Progenics Pharmaceuticals, Inc.)

2.3 Drug Formulation

The proposed marketing formulation is a 150 mg film-coated tablet, each tablet containing 150 mg methylnaltrexone bromide. The inactive ingredients are listed in the following Table (from the Applicant's submission).

Table 1: Comparison of Phase 3 and Proposed Commercial Formulations

Clinical Use [a]	PROPOSED COMMERCIAL FORMULATION	
	MNPK1001 and MNOC1111 (Bridging Studies)	
Manufacturing Process	(b) (4)	
Components (Listed by Functionality)	**Theoretical Quantity**	
	mg/tablet	**% w/w**
Active Ingredient		
Methylnaltrexone bromide	150.00	(b) (4)
(b) (4)		
Silicified microcrystalline cellulose	(b) (4)	
Microcrystalline cellulose		
(b) (4)		
(b) (4)		
Croscarmellose sodium		
Crospovidone (b) (4)		
(b) (4)		
Sodium lauryl sulfate		
(b) (4)		
Poloxamer 407 (b) (4)		
(b) (4)		
Edetate calcium disodium		
(b) (4)		
Talc		
Colloidal silicon dioxide		
(b) (4)		
(b) (4)		
Stearic acid		
(b) (4)		
(b) (4)		
(b) (4)		
(b) (4)		
Total Theoretical Weight	533.59	100.0

[a] Refer to Attachment 3 ((b) (4) and Attachment 4 ((b) (4) for a complete list of clinical studies in which these formulations were used.

[b] Tablets used in phase 3 study MNTX 3201 were uncoated. Coated and uncoated tablets using the same (b) (4) formulation were compared in MNPK 1118, a phase 1 pharmacokinetic study. Uncoated tablets were also used in MNOC1111.

[c] (b) (4)

2.4 Comments on Novel Excipients

No novel excipients are used in the formulation.

2.5 Comments on Impurities/Degradants of Concern

The drug substance, methylnaltrexone bromide, for the proposed 150 mg tablet formulation will be obtained from the currently approved source (Mallinckrodt, Inc.) and (b) (4) The specification for the drug substance is shown in the Applicant's Table below.

Table 1: Drug Substance Specification

Tests	Analytical Procedures/ Methods [a]	Acceptance Criteria
Appearance	Visual	White to off-white crystalline powder
Identification—IR	USP <197K> VR #559	The spectrum of the sample preparation exhibits maxima only at the same wavelengths as that of a similarly prepared reference material.
Identification—HPLC	HPLC VR #814	The retention time of the major peak in the chromatogram of the sample preparation corresponds to that in the chromatogram of the standard preparation obtained in the assay.
Assay	HPLC VR #814	(b) (4)
Related Substances (b) (4)	HPLC VR #814	NMT (b) (4)
		NMT
		NMT
		NMT
		NMT
		NMT
		NMT
		NMT
	HPLC VR #720	NMT
Water Content (Karl Fischer)	SM-29a USP <921> Method I	NMT (b) (4)

Continued

Table 1: Drug Substance Specification (Cont'd)

Tests	Analytical Procedures/ Methods [a]	Acceptance Criteria
Residue on Ignition	SM-199 USP <281> Ph. Eur. 2.4.14	NMT (b) (4)
Loss on Drying	USP <731> Ph. Eur. 2.2.32	NMT (b) (4)
Residual Solvents		
(b) (4)	HPLC VR #576	NMT (b) (4)
(b) (4)	GC VR #486	NMT (b) (4)
Specific Rotation	USP <781S> Ph. Eur. 2.2.20	(b) (4)
(b) (4)		
Solubility (1.5 g in 20 mL of water)	General Physical Property	Clear solution
Color Test (Optical Density)	Spectrophotometry	Report maximum AU
pH	USP <791> Ph. Eur. 2.2.23	(b) (4)
Heavy Metals	USP <231> Method II	NMT (b) (4)
Microbial Limits		
Total Aerobic Microbial Count	USP <61>	NMT (b) (4)
Total Combined Yeasts and Molds Count	USP <61>	NMT
Escherichia coli	USP <62>	(b) (4)
Salmonella species	USP <62>	
Pseudomonas aueruginosa	USP <62>	
Staphylococcus aureus	USP <62>	

Abbreviations: AU= absorbance units, cfu = colony forming units, GC = gas chromatography, HPLC = high-performance liquid chromatography, IR = infrared (spectroscopy), NMT = not more than, Ph. Eur. = European Pharmacopoeia, USP = United States Pharmacopeia, w/w = weight/weight.

[a] The analytical procedures for methylnaltrexone bromide drug substance are described in Mallinckrodt Specifications and Methods Manual ST-DMS-Specification-SMM-10002639 (provided in Drug Master File (b) (4).

[b] (b) (4)

The specification for the drug substance is similar to the specifications approved in NDA 21964 with the exception for residual solvents and specified drug related substances. The specifications for the drug-related impurities in the (b) (4) substance are controlled at levels of NMT (b) (4) % with total impurities at NMT (b) (4) % (see the Table below). These specifications are in accordance with the ICH Q3A (R2) Guidance for drugs with maximum daily doses not exceeding 2 g/day, and are acceptable.

(b) (4)	NMT (b) (4) %
	NMT %
	NMT %
	NMT %
	NMT %
Any Single Unspecified Impurity	NMT %
Total Impurities	NMT %

(b) (4)

contains a structural alert for genotoxicity. However, genotoxicity studies with this

impurity were negative for mutagenicity or clastogenicity. Thus, this impurity was controlled at NMT (b) (4)% as per ICH Q3A (R2), and is acceptable.

The levels of two residual solvents ((b) (4)) in the (b) (4) drug substance were controlled at NMT (b) (4) ppm and (b) (4) ppm, respectively, and are acceptable. The acceptance criteria for these solvents as per ICH Q3C (R5) are (b) (4) ppm and (b) (4) ppm which are (b) (4) than the proposed levels.

The specifications for the drug-related impurities in the Mallinckrodt drug substance is shown in the Table below.

(b) (4)	NMT (b) (4)	w/w
	NMT	w/w
	NMT	w/w
	NMT	w/w
	NMT	w/w
	NMT	w/w
	NMT	w/w
Any Single Unspecified Related Substance	NMT	w/w
Total Related Substances	NMT	/w

These specifications for the drug-related impurities are consistent with the ICH Guidance Q3A (R2) specification limits, and are acceptable.
The acceptance criteria for (b) (4), for the Mallinckrodt drug substance is set at a (b) (4)%) than the (b) (4) drug substance.
The specifications for the two residual solvents, (b) (4) are set at NMT (b) (4) ppm and NMT (b) (4) ppm, respectively. These specifications are in compliance with the ICH Q3C (R5) acceptance levels of (b) (4) ppm and (b) (4) ppm, respectively.

The specification for the drug product, Methylnaltrexone Bromide Oral Tablets, is shown in the Applicant's Table below.

Table 1: Specification for Methylnaltrexone Bromide Tablets, 150 mg

Tests	Analytical Procedures / Methods	Acceptance Criteria
Appearance	QCTP-286 Visual	White to off-white, round, biconvex film-coated tablet, debossed with "REL" on one side and plain on the other side
Identification—HPLC by Retention Time	QCTP-284 HPLC	The retention time of the principal peak in the sample corresponds to the retention time of the principal peak in the reference standard as determined in the test for assay.
Identification—UV by Diode Array Spectrum	QCTP-284 HPLC	The UV spectrum from (b) (4) um of the sample preparation corresponds to the UV spectrum of the reference standard preparation as determined in the test for assay.
Assay	QCTP-284 HPLC	(b) (4) % Label Claim (150 mg methylnaltrexone bromide/tablet)
Impurities [a]	QCTP-288 HPLC	
(b) (4)		NMT (b) (4) % Label Claim
		NMT % Label Claim
Individual Unspecified Impurities		NMT % Label Claim
Total Impurities		NMT % Label Claim
Uniformity of Dosage Units	QCTP-284 USP <905>	Meets USP <905> criteria for content uniformity of film-coated tablets
Dissolution	QCTP-285 USP <711> Apparatus 2	Q = (b) (4) % dissolved in 15 minutes
(b) (4)		

Continued

Table 1: Specification for Methylnaltrexone Bromide Tablets, 150 mg (Cont'd)

Tests	Analytical Procedures / Methods	Acceptance Criteria
Microbial Limits	MM-1458	
Total Aerobic Microbial Count	USP <61> and USP <62>	NMT (b) (4) cfu/g
Total Combined Yeasts and Molds Count		NMT (b) (4) cfu/g
Escherichia coli		(b) (4)

Abbreviations: cfu = colony forming units, HPLC = high-performance liquid chromatography, NMT = not more than, USP = United States Pharmacopeia, UV = ultraviolet (spectroscopy).

[a] (b) (4)

A forced degradation study was conducted with Methylnaltrexone Bromide Tablets, 150 mg to determine the impurities present in the drug product under stress conditions. Structural characterizations of majority of the impurities have been provided in NDA 21964 for Relistor SC Injection with the exception of the following three degradants: (b) (4)

[redacted] The levels of [redacted] was [redacted]% for all batches on long-term and accelerated stability testing. Higher levels of this impurity have only been observed under base stress conditions.

[redacted] Low levels (up to [redacted]%) of this impurity have been observed on long-term and accelerated storage, and higher levels have been observed under acid stress conditions. The levels of [redacted] in the drug product are acceptable at the levels specified.

During the long-term stability studies with the original formulation of 150 mg Methylnaltrexone Bromide Tablets, an unknown impurity was observed at relative retention time of [redacted] in the HPLC/UV chromatograms. The impurity has been identified as [redacted] which is similar to the known impurity, [redacted] eported in NDA 21964 for SC Relistor.

Two potential impurities, [redacted] are controlled at levels of not more than (NMT) [redacted]%, which is consistent with ICHQ3B(R2) qualification threshold for impurities when the maximum daily dose of the drug substance is 100 mg to 2.0 g.

2.6 Proposed Clinical Population and Dosing Regimen

Methylnaltrexone bromide tablets are indicated for the treatment of opioid induced constipation in adult patients with chronic non-cancer pain. The recommended dosing regimen is 3 tablets of 150 mg once daily.

2.7 Regulatory Background

N/A

3 Studies Submitted

3.1 Studies Reviewed

Pharmacokinetic study assessing the binding affinities of methylnaltrexone and its metabolites, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone on human μ-, κ- and δ-Opioid receptors (Study # AB22501; study dates Nov 21, 2013 to Dec 02, 2013).

Evaluation of the effects of methyl-6α-naltrexol, methyl-6β-naltrexol and methylnaltrexone sulfate on cloned HERG channels expressed in human embryonic kidney (HEK293) cells (Study No. MNIV0105, April 2015).

In vitro assessment of methylnaltrexone sulfate, methy-6α-naltrexol, and methyl-6β-naltrexol protein binding in human plasma (Protocol XS-0641; Study initiation date, January 30, 2015).

Title: Evaluation of the potential for direct glucuronidation of n-methylnaltrexone (MNTX) in human, Sprague-Dawley rat, and beagle dog microsomes ((b) (4) Project No. 107N-0407, 2004)

Identification of Cytochrome P450 drug-metabolizing enzymes involved in the metabolism of N-methylnaltrexone (MOA-728; MNTX) utilizing cDNA-expressed human enzymes ((b) (4) Study # 7434-113, 2005)

Evaluation of Inhibitory Potential of Methylnaltrexone (MNTX) Against Six Principal Human Cytochrome P450 Enzymes Using Human Hepatic Microsomes ((b) (4) Project No. 107N-0304, 2004)

In Vitro Evaluation of Methylnaltrexone Sulfate, Methyl-6α-Naltrexol and Methyl-6β-Naltrexol as Inhibitors and Substrates of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K (Study # XS-0621/ XT148071; Non-GLP; Study initiation date: December 01, 2014)

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

Pharmacology/Toxicology review of NDA 21964 for Relistor Injection.

4 Pharmacology

4.1 Primary Pharmacology

In a pharmacodynamic study (Study # AB22501; study dates Nov 21, 2013 to Dec 02, 2013; (b) (4) the binding affinities of methylnaltrexone and its metabolites, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone on human μ-, κ- and δ-Opioid receptors was assessed *in vitro*.

Methylnaltrexone and its metabolites were found to bind with μ- receptors with higher affinities (IC_{50} values, 34 nM to 10.7 μM). The binding affinities for the κ- receptors were similar (IC_{50} values, 0.51 to 0.59 μM), while the IC_{50} values for the δ- receptors were >30 μM. The binding affinities (IC_{50} and K_i values) for methylnaltrexone, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone on human μ-, κ- and δ-Opioid receptors are summarized in the Applicant's Table below.

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}*	K_i	n_H
Compound: Methyl-6-alpha-Naltrexol, PT #: 1177131							
260130	Opiate δ_1 (OP1, DOP)	hum	37 µM	53	32.6 µM	5.61 µM	1.13
260210	Opiate κ(OP2, KOP)	hum	1.2 µM	72	0.51 µM	0.21 µM	0.84
260410	Opiate µ(OP3, MOP)	hum	0.037 µM	52	0.034 µM	0.014 µM	0.75
Compound: Methyl-6-beta-Naltrexol, PT #: 1177132							
260130	Opiate δ_1 (OP1, DOP)	hum	100 µM	66	45.6 µM	7.85 µM	0.92
260210	Opiate κ(OP2, KOP)	hum	1 µM	61	0.59 µM	0.24 µM	0.82
260410	Opiate µ(OP3, MOP)	hum	0.3 µM	63	0.19 µM	0.078 µM	0.98
Compound: Methylnaltrexone, PT #: 1176989							
260130	Opiate δ_1 (OP1, DOP)	hum	37 µM	54	33.1 µM	5.69 µM	1.11
260210	Opiate κ(OP2, KOP)	hum	1.2 µM	71	0.51 µM	0.20 µM	1.02
260410	Opiate µ(OP3, MOP)	hum	0.12 µM	56	0.10 µM	0.042 µM	0.91
Compound: Methylnaltrexone-3-sulfate, PT #: 1177134							
260210	Opiate κ(OP2, KOP)	hum	25 µM	55	21.4 µM	8.56 µM	0.69
260410	Opiate µ(OP3, MOP)	hum	25 µM	71	10.7 µM	4.33 µM	1.09

4.2 Secondary Pharmacology

No secondary pharmacology study reports were submitted.

4.3 Safety Pharmacology

Study Title: Evaluation of the effects of methyl-6α-naltrexol, methyl-6β-naltrexol and methylnaltrexone sulfate on cloned HERG channels expressed in human embryonic kidney (HEK293) cells.

Study No: 1310-031 (b) (4); Sponsor Study No. MNIV0105
Testing facility: (b) (4)
Date of Study Initiation/Termination: April 10, 2015/April 21, 2015
GLP Compliance - Yes

Methods: The study was conducted to assess the effects of methyl-6α-naltrexol, methyl-6β-naltrexol and methylnaltrexone sulfate on the rapidly activating inward rectifying potassium current (Ikr) in cloned hERG channels expressed in human embryonic kidney (HEK293) cells. The concentrations used were 0.1, 1, 10 and 100 µM for methyl-6α-naltrexol, methyl-6β-naltrexol and methylnaltrexone sulfate. Cisapride was used as the positive control at a concentration of 0.1 µM, and physiological saline solution (PSS) was used as a negative control. Currents were recorded from HEK293 cells (at least 3 cells at each concentration) expressing hERG using whole-cell patch clamp technique.

Results: Treatment of the cells with the vehicle (PSS) did not have an effect on the mean hERG mediated potassium currents. Treatment with the test agents, methyl-6α-naltrexol, methyl-6β-naltrexol and methylnaltrexone sulfate, at concentrations up to 100 µM, had no significant effect on hERG currents, while the positive control cisapride (0.1 µM) produced about 80% inhibition of the hERG currents in HEK293 cells.
The findings are summarized in the Applicant's Table below.

Summary of hERG Current Inhibition, %

Concentration	Inhibition, % Mean	SEM	N
0 µM Vehicle (PSS)	-0.12	0.470	5
0.1 µM Methylnaltrexone sulfate	-0.18	1.265	3
1 µM Methylnaltrexone sulfate	0.93	0.367	3
10 µM Methylnaltrexone sulfate	0.58	0.162	3
100 µM Methylnaltrexone sulfate	2.37	1.193	4
0.1 µM Methyl-6α-naltrexol	1.74	0.904	3
1 µM Methyl-6α-naltrexol	1.37	1.184	4
10 µM Methyl-6α-naltrexol	-0.91	0.351	3
100 µM Methyl-6α-naltrexol	0.69	0.130	3
0.1 µM Methyl-6ß-naltrexol	0.53	1.256	3
1 µM Methyl-6ß-naltrexol	0.87	0.314	3
10 µM Methyl-6ß-naltrexol	-1.25	1.338	3
100 µM Methyl-6ß-naltrexol	0.94	0.583	4
0.1 µM Cisapride	79.53	0.631	4

N - Number of measures used to calculate mean
SEM - Standard Error of the Mean

Thus, methyl-6α-naltrexol, methyl-6β-naltrexol and methylnaltrexone sulfate no inhibitory effect on hERG-mediated potassium currents in HEK293 cells at concentrations up to 100 µM. The highest concentration of the test articles tested is more than 300 times the expected maximum free plasma concentration at the therapeutic dose of 450 mg/day.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study Title: In vitro assessment of methylnaltrexone sulfate, methy-6α-naltrexol, and methyl-6β-naltrexol protein binding in human plasma.

Methods: The study was conducted at (b) (4) Protocol XS-0641; Study initiation date, Jan 30, 2015) to assess the in vitro protein binding of methylnaltrexone sulfate, methy-6α-naltrexol, and methyl-6β-naltrexol in human plasma by the equilibrium dialysis method. Methylnaltrexone sulfate (Lot # 12-OC-FP-081), methy-6α-naltrexol (Lot # 12-OC-FP-080), and methyl-6β-naltrexol (Lot # 12-OC-FP-096) were dissolved in DMSO (1 mg/mL conc.) and further diluted in DMSO prior to incubating with pooled human plasma. Plasma was collected from three healthy male subjects under fasted condition. The final concentrations of the test agents used were 1, 10 and 50 ng/mL. Warfarin (10 µmol/L) was used as a positive control.

Results: In vitro plasma protein bindings at 1, 10 and 50 ng/ml concentrations were 17.7%, 17.3% and 28.9% for methylnaltrexone sulfate, 29.4%, 20.8% and 24.3% for methy-6α-naltrexol, and 41.3%, 30.3% and 31.4% for methyl-6β-naltrexol, respectively. The protein binding of the control substance, warfarin was 98.8%. The plasma protein binding data for methylnaltrexone sulfate, methy-6α-naltrexol, and methyl-6β-naltrexol are shown in the Applicant's Table below.

Table 1 Plasma protein binding of methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol in human plasma

Test article	concentration (ng/mL)	Unbound ratio (%)		Protein binding ratio (%)		Recovery (%)	
		Individual	Mean ± SD	Individual	Mean ± SD	Individual	Mean ± SD
methylnaltrexone sulfate	1	84.9	82.3 ± 13.7	15.1	17.7 ± 13.7	100.0	95.1 ± 4.6
		67.4		32.6		91.0	
		94.5		5.5		94.3	
	10	71.3	82.7 ± 13.2	28.7	17.3 ± 13.2	81.0	86.6 ± 5.4
		97.1		2.9		91.7	
		79.6		20.4		87.0	
	50	60.3	71.1 ± 9.6	39.7	28.9 ± 9.6	90.1	97.6 ± 6.8
		78.5		21.5		99.5	
		74.5		25.5		103.2	
methyl-6α-naltrexol	1	66.2	70.6 ± 4.4	33.8	29.4 ± 4.4	95.0	95.9 ± 4.6
		75.0		25.0		91.9	
		70.7		29.3		100.9	
	10	80.6	79.2 ± 4.8	19.4	20.8 ± 4.8	85.9	95.0 ± 8.2
		83.1		16.9		97.4	
		73.9		26.1		101.8	
	50	82.3	75.7 ± 6.5	17.7	24.3 ± 6.5	84.6	96.1 ± 10.0
		75.3		24.7		100.9	
		69.4		30.6		102.8	
methyl-6β-naltrexol	1	54.0	58.7 ± 5.0	46.0	41.3 ± 5.0	90.7	86.8 ± 3.7
		58.2		41.8		83.4	
		63.9		36.1		86.2	
	10	84.0	69.7 ± 12.4	16.0	30.3 ± 12.4	81.3	83.0 ± 3.6
		63.1		36.9		87.2	
		62.0		38.0		80.6	
	50	63.3	68.6 ± 6.9	36.7	31.4 ± 6.9	91.2	91.4 ± 0.7
		76.4		23.6		92.2	
		66.0		34.0		90.8	

Thus, the plasma protein binding of methylnaltrexone sulfate, methy-6α-naltrexol, and methyl-6β-naltrexol was low in human plasma.

Metabolism:

Study Title: Evaluation of the potential for direct glucuronidation of n-methylnaltrexone (MNTX) in human, Sprague-Dawley rat, and beagle dog microsomes ((b) (4) Project No. 107N-0407, 2004)

Methods: Glucuronidation of N-methylnaltrexone (MOA-728; MNTX) was assessed by incubating [^{14}C]MOA-728 with liver microsomes of rats, dogs and humans at 37^{0}C. [^{14}C]MOA-728 was incubated with the liver microsomes at 1.15 and 11.5 µM concentrations for 0, 15, 30 and 60 minutes in the presence of UDPGA (5 mM; total incubation volume 500 µL). At the end of the incubation, the samples were analyzed by LC/MS/MS after addition of [^{13}CD3]MOA-728 as an internal standard. Oxymorphone (20 µM) was used as a positive control, and the negative control incubations were carried out without UDPGA in duplicate at a MNTX concentration of 0.5 µg/mL.

Results: Following incubation of [^{14}C]MOA-728 with liver microsomes, the formation of MOA-728 glucuronide in liver microsomes of rats and dogs was concentration and time dependent. Rat live microsomes produced higher glucuronidation than dog or human microsomes. Formation of MOA-728 glucuronide following incubation of [^{14}C]MOA-728 with human microsomes was very low, and was detected only at 60 minutes. Formation MNTX glucuronide with rat microsomes was 22.8-fold higher than that observed with dog microsomes (based on mean peak area ratios at 60 minutes). The mean peak area ratios for MNTX and MNTX glucuronide for rat, dog and human liver microsomes at different periods of incubation are shown in the Applicant's Table below.

Table 1. Formation of MNTX-Glucuronide in Rat, Dog, and Human Hepatic Microsomal Incubations

Mean Peak Area Ratio	MNTX (355.9>284.1)			
Incubation Time (minute)	0	15	30	60
MNTX 0.5 µg/mL, Rat Microsomes	71.505	72.300	75.774	72.359
MNTX 5.0 µg/mL, Rat Microsomes	328.619	323.905	331.411	325.016
MNTX 0.5 µg/mL, Dog Microsomes	76.574	78.150	78.780	79.727
MNTX 5.0 µg/mL, Dog Microsomes	350.117	355.749	372.329	362.060
MNTX 0.5 µg/mL, Human Microsomes	56.513	54.898	56.520	57.160
MNTX 5.0 µg/mL, Human Microsomes	249.134	247.551	252.640	259.435

Mean Peak Area Ratio	MNTX-Glucuronide (532>532)			
Incubation Time (minute)	0	15	30	60
MNTX 0.5 µg/mL, Rat Microsomes	0.000	0.320	0.667	0.693
MNTX 5.0 µg/mL, Rat Microsomes	0.000	1.806	3.713	7.295
MNTX 0.5 µg/mL, Dog Microsomes	0.000	0.000	0.000	0.000
MNTX 5.0 µg/mL, Dog Microsomes	0.000	0.000	0.243	0.347
MNTX 0.5 µg/mL, Human Microsomes	0.004	0.000	0.000	0.010
MNTX 5.0 µg/mL, Human Microsomes	0.000	0.000	0.000	0.000

Mean Peak Area Ratio	MNTX-Glucuronide (532>355.9)			
Incubation Time (minute)	0	15	30	60
MNTX 0.5 µg/mL, Rat Microsomes	0.000	0.065	0.131	0.137
MNTX 5.0 µg/mL, Rat Microsomes	0.000	0.345	0.718	1.392
MNTX 0.5 µg/mL, Dog Microsomes	0.000	0.003	0.004	0.008
MNTX 5.0 µg/mL, Dog Microsomes	0.000	0.016	0.033	0.061
MNTX 0.5 µg/mL, Human Microsomes	0.000	0.000	0.000	0.000
MNTX 5.0 µg/mL, Human Microsomes	0.000	0.000	0.001	0.001

Thus, the direct glucuronide conjugation of N-methylnaltrexone following *in vitro* incubation with liver microsomes of rats, dogs and humans varied across the species examined. Rat liver microsomes had higher activity than dog liver microsomes, and human liver microsomes showed very low activity.

Study Title: Identification of Cytochrome P450 drug-metabolizing enzymes involved in the metabolism of N-methylnaltrexone (MOA-728; MNTX) utilizing cDNA-expressed human enzymes ((b) (4) Study # 7434-113, 2005)

Methods: The objective of the study was to identify the CYP450 enzymes involved in the metabolism of MOA-728. [^{14}C]MOA-728 was incubated at 37^{0}C for 30 minutes with cDNA-expressed human CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and mixed enzyme pool in the presence of 1 mM NADPH. The concentrations of [^{14}C]MOA-728 used were 0.28, 2.8 and 28 µM. The metabolites were analyzed by HPLC with radiochemical detection.

Results: The mean sample extraction efficiencies were 131%, 116% and 84% for 0.28, 2.8 and 28 µM concentrations of MNTX, respectively. No metabolism of radiolabeled MNTX was observed following incubation with any of the recombinant enzymes or the enzyme pool under the conditions of the study. Only a single peak of MNTX was identified in the radiochromatogram for all incubation samples. The study results indicate that MNTX does not undergo CYP450-mediated metabolism.

Study Title: Evaluation of Inhibitory Potential of Methylnaltrexone (MNTX) Against Six Principal Human Cytochrome P450 Enzymes Using Human Hepatic Microsomes ((b) (4) Project No. 107N-0304, 2004)

Methods: The potential inhibitory effects of MOA-728 (methylnaltrexone; MXTX; Batch No. E06275; purity 98.5%) on cytochrome P450 activities of six CYP enzymes (CYP1A2, CYP1A6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were assessed in pooled hepatic microsomes using phenacetin, coumarin, tolbutamide, S-mephenetoin, dextromethorphan, midazolam and testosterone, respectively as the enzyme substrates. The metabolites of the enzyme substrates (acetaminophen, 7-hydroxycoumarin, 4-hydroxytolbutamide, 4'-hydroxymephenytoin, dextrorphan, 1'-hydroxymidazolam and 6β-hydroxytestosterone, respectively) were measured using an LC/MS/MS method. The positive control substrates used in the assay are summarized in the Table below (from the applicant's submission).

Summary of CYP450 Substrates, Metabolites, and Positive Controls

CYP Isoform	Substrate	Final Concentration of Substrate	Metabolite Analyzed	Positive Control Inhibitor	Final Highest Concentration of Positive Control
1A2	Phenacetin	30 µM	Acetaminophen	Furafylline	100 µM
2A6	Coumarin	2.5 µM	7-hydroxycoumarin	Tranylcypromine	10 µM
2C9	Tolbutamide	150 µM	4-hydroxytolbutamide	Sulfaphenazole	10 µM
2C19	S-Mephenytoin	100 µM	4'-hydroxymephenytoin	Omeprazole	100 µM
2D6	Dextromethorphan	8 µM	Dextrorphan	Quinidine	10 µM
3A4	Testosterone	50 µM	6β-hydroxytestosterone	Ketoconazole	10 µM
3A4	Midazolam	25 µM	1'-hydroxymidazolam	Ketoconazole	10 µM

Results: MNTX did not inhibit CYP1A2, CYP2A6, CYP2C9, CYP2C19 and CYP3A4 using selected probe substrates for these enzymes. However, MNTX caused an inhibition of CYP2D6 activity in human hepatic microsomes with an IC_{50} value of 15.92 µM. The IC_{50} values for the positive controls were 2.676, 0.618, 0.326, 22.271, 0.098, 0.358 and 0.415 µM for CYP1A2, CYP1A6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, respectively. Following incubation of five concentrations (2, 4, 8, 16 and 32 µM) of the CYP2D6 substrate, dextromethorphan with 5 and 10 µM MNTX, it was found that MNTX is a competitive inhibitor of CYP2D6 in human microsomes with an inhibition constant (Ki) of 7.93 µM. CYP enzyme inhibitory activity for different enzymes by MNTX is shown the Applicant's table below.

. **Summary of Inhibition of CYP450 Activity by MNTX**

	CYP450 Activity Expressed as % Vehicle Control						
MNTX Concentration (µM)	CYP1A2	CYP2A6	CYP2C9	CYP2C19	CYP2D6	CYP3A4/5	CYP3A4/5
100	75	98	96	108	18	109	110
33.3	72	101	96	108	36	109	102
11.1	75	99	93	102	58	103	98
3.70	80	99	97	105	76	102	95
1.23	96	101	107	109	95	110	105
0.41	89	99	109	112	101	110	104
0.137	87	100	109	106	97	106	105
0.046	90	96	105	111	104	107	107
0.015	87	99	101	106	97	101	95
0 (VC)	100	100	100	100	100	100	100
IC_{50} (µM)	> 100 µM	> 100 µM	> 100 µM	> 100 µM	15.92 µM	> 100 µM	> 100 µM
Substrate	Phenacetin	Coumarin	Tolbutamide	S-Mephenytoin	Dextromethorphan	Midazolam	Testosterone

VC = Vehicle Control.

In summary, MNTX did not cause inhibition of CYP1A2, CYP2A6, CYP2C9, CYP2C19 and CYP3A4 activity in human hepatic microsomes at concentrations up to 100 µM. MNTX inhibited CYP2D6 activity with an IC_{50} value of 15.92 µM.

Study Title: In Vitro Evaluation of Methylnaltrexone Sulfate, Methyl-6α-Naltrexol and Methyl-6β-Naltrexol as Inhibitors and Substrates of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K (Study # XS-0621/ XT148071; Non-GLP; Study initiation date: December 01, 2014)

Methods:
The study was conducted to evaluate methylnaltrexone, methylnaltrexone sulfate, methyl-6α-naltrexol and methy-6β-naltrexol as inhibitors and substrates of human transporters. The ability of these compounds to inhibit human ATP binding cassette (ABC) transporters (efflux transporters) p-gp and BCRP was assessed using the bidirectional permeability of a probe substrate across a monolayer of Caco-2 or MDCKII-BCRP cells. The ability of methylnaltrexone to inhibit the human solute-linked carrier (SLC) transporters OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1, and MATE2-K and the ability of methylnaltrexone sulfate, methyl-6α-naltrexol and methyl-6β-naltrexol to inhibit human SLC transporters, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1, and MATE2-K was assessed by measuring the accumulation of probe substrates in transporter-expressing and control HEK293 cells in the presence of each test article. Known inhibitors were used as positive controls in all experiments. The study design is summarized in the table below (from the Applicant's submission).

Test articles	Transporter	Test system	Probe substrate	Experimental design
Methylnaltrexone sulfate Methyl-6α-naltrexol Methyl-6β-naltrexol	P-gp	Caco-2	Digoxin	Bidirectional transport of the probe substrate across Caco-2 cells
	BCRP	MDCKII-BCRP	Prazosin	Bidirectional transport of the probe substrate across MDCKII-BCRP and control MDCKII cells
	MRP2	Vesicles	Estradiol-17β-glucuronide	Uptake of the probe substrate into MRP2 vesicles in the presence and absence of ATP
Methylnaltrexone Methylnaltrexone sulfate Methyl-6α-naltrexol Methyl-6β-naltrexol	OATP1B1	HEK293	Estradiol-17β-glucuronide	Uptake of the probe substrate into OATP1B1 and control cells
	OATP1B3	HEK293	Estradiol-17β-glucuronide	Uptake of the probe substrate into OATP1B3 and control cells
	OCT1	HEK293	Tetraethylammonium bromide	Uptake of the probe substrate into OCT1 and control cells (**Note**: inhibition not tested with methylnaltrexone for this transporter)
	OCT2	HEK293	Metformin	Uptake of the probe substrate into OCT2 and control cells
	OAT1	HEK293	*p*-Aminohippurate	Uptake of the probe substrate into OAT1 and control cells
	OAT3	HEK293	Estrone-3-sulfate	Uptake of the probe substrate into OAT3 and control cells
	MATE1	HEK293	Metformin	Uptake of the probe substrate into MATE1 and control cells
	MATE2-K	HEK293	Metformin	Uptake of the probe substrate into MATE2-K and control cells

The table below (from the Applicant's submission) shows the concentrations for each test article used in the study.

Transporter	Methylnaltrexone	Methylnaltrexone sulfate	Methyl-6α-naltrexol	Methyl-6β-naltrexol
P-gp	Not applicable	0.03, 0.1, 0.3, 1, 3, 10 µM	0.03, 0.1, 0.3, 1, 3, 10 µM	0.03, 0.1, 0.3, 1, 3, 10 µM
BCRP				
MRP2		0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM
OATP1B1 / OATP1B3	3, 10, 30, 300, 1000, 1800 µM	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM
OCT1	Not applicable			
OCT2	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM			
OAT1				
OAT3				
MATE1				
MATE2-K				

Results:

Methylnaltrexone:
In the presence of methylnaltrexone, the inhibition of OATP1B3, OAT3, OCT2 and MATE2-K mediated transport did not exceed 15%. There was 16, 48 and 43% inhibition of OATP1B1, OAT1 and MATE1mediated transport, respectively, by methylnaltrexone. An IC_{50} value could not be calculated because none of the inhibition reached 50%.

The uptake ratio of methylnaltrexone in MATE1, MATE@-K, and OCT2-transfected cells was >2 and was reduced to <2 in the presence of the positive control inhibitor. The findings suggest that methylnaltrexone is a substrate for MATE1, MATE2-K and OCT2.

Methylnaltrexone sulfate:

In the presence of methylnaltrexone sulfate, the inhibition of BCRP, P-gp, OATP1B3, OCT1, OCT2, OAT1, MATE1, MATE2-K and MRP2 mediated transport was <15%. OARP1B1 and OAT3 mediated transport was inhibited by methylnaltrexone sulfate by up to 17 and 34%, respectively. In the absence of 50% inhibition of these transporters, an IC_{50} value could not be calculated. The efflux ratio of methylnaltrexone sulfate in MDCKII-BCRP was >2 at 0.3 μM concentration and was reduced to below 2 in the presence of positive control inhibitor which suggest that methylnaltrexone sulfate is a potential substrate of BCRP. Methylnatrexone sulfate was also identified as a substrate for MATE2-K in this study.

Methyl-6α-naltrexol:

In the presence of methyl-6α-naltrexol, MRP2, OATP1B1, OCT1, OCT2, OAT1 and MATE1 mediated transport was inhibited by up to 23, 23, 19, 30, 24 and 16%, respectively. An IC_{50} value could not be determined, because none of the inhibitions exceeded 50%. Methyl-6α-naltrexol was not a substrate for P-gp; however, it may be a substrate for BCRP. Methyl-6α-naltrexol was also identified as a substrate for OCT1, OCT2, MATE1 and MATE2-K.

Methyl-6β-naltrexol:
No concentration-dependent inhibition of P-gp was observed in the presence of methyl-6β-naltrexol. BCRP, OCT1 and MATE1 mediated transport was inhibited up to 29, 48 and 33%, respectively, in the presence of methyl-6β-naltrexol; however, an IC_{50} value could not be determined because none of the inhibitions exceeded 50%. Methyl-6β-naltrexol was not a substrate of BCRP or MRP2, OATP1B1, OATP1B3, OAT1 and OAT3. Methyl-6β-naltrexol was an inhibitor of MATE1, MATE2-K, OCT1, and OCT2 in this study.

The effects of methylnaltrexone, methylnaltrexone sulfate, methyl-6α-naltrexol and methy-6β-naltrexol on different transporters are summarized in the Tables below (from the Applicant's submission).

Transporter	Methylnaltrexone	Methylnaltrexone sulfate	Methyl-6α-naltrexol	Methyl-6β-naltrexol
	Inhibition results: IC_{50} (μM)			
P-gp	NT	> 10	> 10	> 10
BCRP	NT	> 10	> 10	> 10
MRP2	NT	> 10	> 10	> 10
OATP1B1	> 1800	> 10	> 10	> 10
OATP1B3	> 1800	> 10	> 10	> 10
OCT1	NT	> 10	> 10	> 10
OCT2	> 10	> 10	> 10	> 10
OAT1	> 10	> 10	> 10	> 10
OAT3	> 10	> 10	> 10	> 10
MATE1	> 10	> 10	> 10	> 10
MATE2-K	> 10	> 10	> 10	> 10
Transporter	**Methylnaltrexone**	**Methylnaltrexone sulfate**	**Methyl-6α-naltrexol**	**Methyl-6β-naltrexol**
	Maximum percent inhibition (%)			
P-gp	NT	<15%	<15%	<15%
BCRP	NT	<15%	<15%	**29%**
MRP2	NT	<15%	**23%**	<15%
OATP1B1	**16%**	**17%**	**23%**	<15%
OATP1B3	<15%	<15%	<15%	<15%
OCT1	NT	<15%	**19%**	**48%**
OCT2	<15%	<15%	**30%**	≤15%
OAT1	**48%**	<15%	**24%**	<15%
OAT3	<15%	**34%**	<15%	<15%
MATE1	**43%**	<15%	**16%**	**33%**
MATE2-K	<15%	<15%	<15%	<15%

Table continued on next page.

Transporter	Methylnaltrexone	Methylnaltrexone sulfate	Methyl-6α-naltrexol	Methyl-6β-naltrexol
	Substrate results: Is the compound a potential substrate of the transporter (Yes, No)			
P-gp	NT	No	No	No
BCRP	NT	Yes	Yes	No
MRP2	NT	No	No	No
OATP1B1	NT	No	No	No
OATP1B3	NT	No	No	No
OCT1	NT	No	Yes	Yes
OCT2	Yes	No	Yes	Yes
OAT1	NT	No	No	No
OAT3	NT	No	No	No
MATE1	Yes	No	Yes	Yes
MATE2-K	Yes	Yes	Yes	Yes

NT Not Tested

Bolded results indicate maximum percent inhibition greater than 15%.

Thus, methyl naltrexone is a substrate for OCT2, MATE1 and MATE2-K; methylnaltrexone sulfate is a substrate for BCRP, and MATE2-K; methyl-6α-naltrexol is a substrate for BCRP, OCT1, OCT2, MATE1 and MATR2-K; and methy-6β-naltrexol is a substrate for OCT1, OCT2, OAT1, MATE1 and MATE2-K.

6 General Toxicology

6.1 Single-Dose Toxicity

No study was submitted.

6.2 Repeat-Dose Toxicity:

No study was submitted.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

No study was submitted.

7.2 *In Vitro* Assays in Mammalian Cells

No study was submitted.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

No in vivo clastogenicity study was submitted in the current NDA.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

No carcinogenicity studies were submitted in the NDA. However, carcinogenicity studies of methylnaltrexone were reviewed under NDA 21964.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No studies were submitted in this NDA.

9.2 Embryonic Fetal Development

No studies were submitted in the current NDA.

9.3 Prenatal and Postnatal Development

None.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Methylnaltrexone bromide (MNTX, MOA-728) is a peripherally active selective mu-opioid receptor antagonist. Subcutaneous injections of MNTX (Relistor) is currently approved for the treatment of adult patients with advanced illness with opioid-induced constipation (OIC), who are receiving palliative care, and for the treatment of OIC in adult patients with non-cancer pain. In the current NDA, the Applicant is seeking an approval of methylnaltrexone bromide oral tablets (150 mg) for the treatment of OIC in adult patients with chronic non-cancer pain. The recommended oral dose is 3 tablets of 150 mg (a total of 450 mg) a day.

Nonclinical studies, including pharmacology, safety pharmacology, pharmacokinetics, metabolism and toxicology (general toxicology, genetic toxicology, carcinogenicity and reproductive toxicity) studies were submitted and reviewed under NDA 21964 for the approval of subcutaneous Relistor. Methylnaltrexone and its metabolites were found to bind with μ- opioid receptors with high affinities (IC_{50} values, 34 nM to 10.7 μM). In an in vitro hERG assay in human embryonic kidney (HEK293) cells, methylnaltrexone and its metabolites had no significant effect on hERG currents at concentrations up to 100 μM. In an *in vitro* plasma protein binding assay in human plasma, the binding of methylnaltrexone sulfate, 6-alpha-methylnaltrexol, 6-beta-methylnaltrexol and 3-sulfo-methylnaltrexone was relatively low (17% to 41%). Methylnaltrexone was not found to undergo CYP450-mediated metabolism in vitro; however, it was found to be a competitive inhibitor of CYP2D6.

No new toxicology studies of methylnaltrexone were submitted in the current NDA. Toxicology studies submitted in the initial NDA 21964 and sNDA 21964 were reviewed earlier by Dr. Tamal Chakraborti (March 16, 2012). Repeat-dose toxicity studies by the oral route was conducted in mice (up to 90 days), in rats (up to six months), and in dogs (up to 9 months). In addition, the full battery of genotoxicity and reproductive and developmental toxicity studies were conducted with methylnaltrexone bromide, and were reviewed under NDA 21964. Two year carcinogenicity studies in rats and mice were also reviewed earlier (NDA 21964).

In a 90-day oral toxicity study in mice (Study no.7434-101), the animals were treated with methylnaltrexone at 80, 400 and 2000 mg/kg/day doses by oral gavage. There were several mortalities at 2000 mg/kg/day, and the dose was reduced to 1500 mg/kg/day. Increased food consumption was observed at mid and high doses. The 400 mg/kg/day dose was the NOAEL. A target organ could not be identified in the absence

of any significant histopathology findings in any organ or tissue. The NOAEL was considered to be 80 mg/kg/day.

In a six-month repeat-dose oral toxicity study in rats, mortality was observed at 500 mg/kg and higher doses. The cause of the deaths was unclear. The dead animals exhibited moderate to severe congestion in lungs, liver, adrenals and kidneys. The body weights of the high dose (3000/2000/1000 mg/kg/day) animals were decreased by 18% at the end of the treatment period. The NOAEL was considered to be 100 mg/kg/day. At the 100 mg/kg dose, the mean total systemic exposures (AUC0-24) to MNTX were 552 ng.h/mL in males and 667 ng.h/mL in females. At ≥300 mg/kg/day, globule leukocyte/eosinophilic inflammation in the intestine, increased cellularity in the mesenteric lymph nodes, and epithelial degeneration and edema in the squamous mucosa of the stomach were noted microscopically. The 100 mg/kg NOAEL dose in rats is about 13 times the clinical dose of 450 mg/day.

In a 9-month repeated dose oral toxicity study in dogs, the animals were dosed at 20, 60 and 180 mg/kg/day doses. At the beginning of the study, the 180 mg/kg/day dose was increased to 225 mg/kg/day, and subsequently to 250 mg/kg/day by the end of the study. Two female dogs died of apparent gavage errors. Clinical signs included tremor, ataxia, third eyelid elevation, red sclera, and loss of menstrual cycle at mid and high doses. There were no significant treatment-related effects on body weights, food consumption, ophthalmic findings, hematology, clinical chemistry and urinalysis values, organ weights, or macroscopic and microscopic observations. No treatment related effects on blood pressures, HR or ECG parameters were observed at any doses. No target organ of toxicity was identified in the absence of any histopathology changes. The 60 mg/kg/day dose was considered the NOAEL, and this dose is approximately 8 times the proposed clinical dose of 450 mg/day.

Methylnaltrexone bromide was negative in the Ames test, chromosome aberration tests in Chinese hamster ovary cells and human lymphocytes, in the mouse lymphoma cell forward mutation tests and in the *in vivo* mouse micronucleus test.

Two-year oral carcinogenicity studies have been conducted with methylnaltrexone in CD-1 mice at doses up to 200 mg/kg/day in males and 400 mg/kg/day in females, and in Sprague Dawley rats at oral doses up to 300 mg/kg/day in males and females. Oral administration of methylnaltrexone for 104 weeks did not produce any neoplasm in mice and rats.

Methylnaltrexone bromide at subcutaneous doses up to 150 mg/kg/day had no adverse effect on fertility and reproductive performance of male and female rats. An embryofetal development study have been performed in rats at intravenous doses up to 25 mg/kg/day, and did not cause any adverse effects on embryofetal development. In rabbits, intravenous doses of methylnaltrexone up to 16 mg/kg/day did not show any embryofetal toxicity. A pre- and postnatal development study in rats showed no evidence of any adverse effect on pre- and postnatal development at subcutaneous doses of methylnaltrexone up to 100 mg/kg/day.

Thus, the nonclinical safety of oral methylnaltrexone bromide has been adequately assessed in a battery of nonclinical studies, reviewed under NDA 21964 for Relistor Injection. The oral NOAEL doses in the chronic 6-month rat and 9-month dog toxicity studies provide adequate safety margins for the proposed clinical dose. No nonclinical approval issues have been identified for the proposed doses for the proposed patient population.

12 Appendix/Attachments

None.

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/s/

SUSHANTA K CHAKDER
03/23/2016