FORMULATIONS FOR PARENTERAL DELIVERY OF COMPOUNDS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present invention claims priority to United States provisional patent application serial number 60/835,574, filed August 4, 2006, the entirety of which is hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Opioids are widely used in patients with advanced cancers and other terminal diseases to lessen suffering. Opioids are narcotic medications that activate opioid receptors located in the central nervous system to relieve pain. Opioids, however, also react with receptors outside of the central nervous system, resulting in side effects including constipation, nausea, vomiting, urinary retention and severe itching. Most notable are the effects in the gastrointestinal tract (GI) where opioids inhibit gastric emptying and propulsive motor activity of the intestine, thereby decreasing the rate of intestinal transit which can produce constipation. The effectiveness of opioids for pain is often limited due to resultant side effects, which can be debilitating and often cause patients to cease use of opioid analgesics.

[0003] In addition to analgesic opioid induced side effects, studies have suggested that endogenous opioid compounds and receptors may also affect activity of the gastrointestinal (GI) tract and may be involved in normal regulation of intestinal motility and mucosal transport of fluids in both animals and man. (Koch, T. R, et al., Digestive Diseases and Sciences 1991, 36, 712-728; Schuller, A.G.P., et al., Society of Neuroscience Abstracts 1998, 24, 524, Reisine, T., and Pasternak, G., Goodman & Gilman's The Pharmacological Basis of Therapeutics Ninth Edition 1996, 521-555 and Bagnol, D., et al., Regul. Pept. 1993, 47, 259-273). Thus, an abnormal physiological level of endogenous compounds and/or receptor activity may lead to bowel dysfunction.

[0004] For example, patients who have undergone surgical procedures, especially surgery of the abdomen, often suffer from bowel dysfunction, such as post-operative (or post-surgical) ileus, that may be caused by fluctuations in natural opioid levels. Similarly, women who have recently given birth commonly suffer from post-partum ileus, which is thought to be

caused by similar natural opioid fluctuations as a result of birthing stress. Bowel dysfunction associated with post-operative or post partum ileus can typically last for 3 to 5 days, with some severe cases lasting more than a week. Administration of opioid analgesics to a patient after surgery, which is now an almost universal practice, may exacerbate bowel dysfunction, thereby delaying recovery of normal bowel function, prolonging hospital stays, and increasing medical care costs.

[0005] Opioid antagonists such as naloxone, naltrexone, and nalmefene, have been studied as a means of antagonizing undesirable peripheral effects of opioids. However, these agents act not only on peripheral opioid receptors, but also on central nervous system sites, so that they sometimes reverse the beneficial analgesic effects of opioids, or cause symptoms of opioid withdrawal. Preferable approaches for use in controlling opioid-induced side effects include use of peripheral opioid antagonist compounds that do not readily cross the blood-brain barrier. For example, the peripheral μ opioid antagonist compound methylnaltrexone and related compounds have been disclosed for use in curbing opioid-induced side effects in patients (e.g., constipation, pruritus, nausea, and/or vomiting). See, e.g., U.S. Pat. Nos. 5,972,954, 5,102,887, 4,861,781, and 4,719,215; and Yuan, C. -S. et al. Drug and Alcohol Dependence 1998, 52, 161.

[0006] Formulations of peripheral μ opioid receptor antagonist methylnaltrexone have been described (e.g., see, for example, U.S. Pat. Nos. 6,608,075, 6,274,591, and 6,559,158). However, methylnaltrexone in certain mediums and under certain conditions has been found to form degradation products. For example, see US 2004266806A1. It is desirable to provide dosage forms that are capable of effective delivery of methylnaltrexone without extensive degradation of the methylnaltrexone under refrigeration and/or room temperature conditions.

SUMMARY OF THE INVENTION

[0007] The present invention provides certain methylnaltrexone formulations. In some embodiments, the invention provides formulations having improved shelf-life stability characteristics of active compound under refrigeration as well as at room temperature conditions. Provided formulations are useful for parenteral administration of methylnaltrexone. The invention includes methods for production and use of such formulations, as well as products and kits containing provided formulations.

[0008] In certain embodiments a pharmaceutical composition is provided containing an effective amount of at least one active compound selected from at least methylnaltrexone, or a pharmaceutically acceptable salt thereof, and a calcium salt chelating agent in an aqueous solution.

[0009] In other embodiments, liquid formulations containing methylnaltrexone, or a pharmaceutically acceptable salt thereof, a calcium salt, a chelating agent, an isotonic agent, and an aqueous solvent are provided. In certain embodiments, a calcium salt and a chelating agent are provided together as a calcium salt chelating agent. In some embodiments, a calcium salt chelating agent is selected from calcium ethylenediaminetetraacetic acid (EDTA), calcium diethylenetriaminepentaacetic acid (DTPA), calcium hydroxyethylenediaminetriacetic acid (HEDTA), calcium ethylene glycol-bis-(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), calcium nitrilotriacetic acid (NTA), calcium citrate, and calcium salt derivatives thereof. In some embodiments a calcium salt chelating agent is calcium EDTA.

[0010] In some embodiments, formulations further comprise an additional stabilizing agent. In some embodiments, a stabilizing agent is selected from glycine, benzoic acid, citric, glycolic, lactic, malic, and maleic acid. In certain embodiments, a stabilizing agent is glycine.

[0011] In certain embodiments, a formulation comprises methylnaltrexone or a pharmaceutically acceptable salt thereof, a calcium chelating agent, a stabilizing agent, an isotonic agent, and an aqueous solvent. In some embodiments, a formulation comprises methylnaltrexone or a pharmaceutically acceptable salt thereof, calcium EDTA, glycine, and sodium chloride, in an aqueous solution.

[0012] In general, provided formulations are useful for preventing, treating or reducing severity of side effects resulting from use of opioids, including inhibition of gastrointestinal dysfunction (e.g., constipation, bowel hypomotility, impaction, gastric hypomotility, GI sphincter constriction, increased sphincter tone, inhibition of gastrointestinal motility, inhibition of intestinal motility, inhibition of gastric emptying, delayed gastric emptying, incomplete evacuation, nausea, emesis (vomiting), bloating, abdominal distension), cutaneous flushing, sweating, dysphoria, pruritis, urinary retention, etc. Provided formulations are useful for administration to patients receiving short term opioid treatment (e.g., patients recovering from surgery (abdominal, orthopedic, surgery from trauma injuries etc.), patients recovering from trauma injuries, and patients recovering from child birth). Formulations are also useful for

administration to subjects receiving chronic opioid administration (e.g., terminally ill patients receiving opioid therapy (e.g., an AIDS patient, a cancer patient, a cardiovascular patient); subjects receiving chronic opioid therapy for pain management (e.g., back pain); subjects receiving opioid therapy for maintenance of opioid withdrawal).

[0013] Additional uses of provided formulations include prevention, treatment or reduction of severity of symptoms associated with disorders or conditions resulting from normal or aberrant activity of endogenous opioids. Such disorders or conditions include, among others, ileus (e.g., post-partum ileus, paralytic ileus), gastrointestinal dysfunction that develops following abdominal surgery (e.g., colectomy, including but not limited to, right hemicolectomy, left hemicolectomy, transverse hemicolectomy, colectomy takedown, and low anterior resection) such as post-operative ileus, and idiopathic constipation. Provided formulations are also useful in treatment of conditions including, for example, cancers involving angiogenesis, inflammatory disorders (e.g., irritable bowel disorder), immune suppression, cardiovascular disorders (e.g., bradycardia, hypotension) chronic inflammation and/or chronic pain, sickle cell anemia, vascular wounds, and retinopathy, decreased biliary secretion, decreased pancreatic secretion, biliary spasm, and increased gastroesophageal reflux.

BRIEF DESCRIPTION OF THE DRAWING

[0014] **Figure 1A** and **Figure 1B**: Effect of CaEDTA and NaEDTA on the formation of 2',2 -bis methylnaltrexone in the presence of iron at 40° C (Figure 1A) and room temperature, 25° (Figure 1B). Both calcium EDTA and sodium EDTA are effective inhibitors of formation of the 2',2' bis methylnaltrexone degradant.

[0015] **Figures 2A, 2B, 2C**, and **2D**: Effect of CaEDTA on the formation of 7-dihydroxy methylnaltrexone in solutions. The effect of CaEDTA and NaEDTA on the formation of 7-dihydroxy methylnaltrexone in the presence of iron at 40° C (Figure 2A) and room temperature, 25° (Figure 2B) was assessed. Calcium EDTA but not sodium EDTA is an effective inhibitor of formation of the 7-dihydroxy-methylnaltrexone degradant. The effect of CaEDTA on the formation of 7-dihydroxy methylnaltrexone in solution following one month storage at room temperature (Figure 2C) and at 40° C (Figure 2D) was assessed. The presence of CaEDTA reduced formation of 7-dihydroxy methylnaltrexone at either temperature. After one month at room temperature, the level was reduced from 0.34% to 0.11%; and at 40° C/75%RH, the level

was reduced from 0.64% to 0.14%. The presence of NaEDTA in the samples may even increase levels of 7-dihydroxy methylnaltrexone formed.

[0016] **Figure 3A** and **Figure 3B**: Effect of CaEDTA in methylnaltrexone solution on the formation of a methylnaltrexone degradant having an RRT 0.79 ("the 0.79 degradant"). The effect of CaEDTA and NaEDTA on the formation of the 0.79 degradant at room temperature, 25° (Figure 3A) and at 40° C (Figure 3B) was assessed. Calcium EDTA was not effective at inhibiting formation of the 0.79 degradant, and may increase levels of degradant formation.

[0017] **Figure 4** depicts identified degradants of methylnaltrexone, respective relative retention times (RRT), and associated catalysis and/or inhibitors of formation which have been identified.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

[0018] Provided are pharmaceutical formulations having improved stability characteristics under certain conditions. Compositions, kits, and products including provided formulations allow for extended storage periods and also for storage under favorable room temperature conditions. Compositions and kits and products containing provided formulations thus allow for improved delivery of therapeutics to subjects benefiting from use of methylnaltrexone.

[0019] For example, provided formulations are useful to treat, prevent, delay, or decrease severity or incidence of side effects associated with opioid administration, including gastrointestinal dysfunction (e.g., constipation, bowel hypomotility, impaction, gastric hypomotility, GI sphincter constriction, increased sphincter tone, inhibition of gastrointestinal motility, inhibition of intestinal motility, inhibition of gastric emptying, delayed gastric emptying, incomplete evacuation, nausea, emesis (vomiting), bloating, abdominal distension), dysphoria, pruritis, urinary retention, depression of respiration, papillary constriction, cardiovascular effects, chest wall rigidity and cough suppression, depression of stress response, and immune suppression associated with use of narcotic analgesia, etc. Additional effects of opioid administration can include, e.g., aberrant migration or proliferation of endothelial cells (e.g., vascular endothelial cells), increased angiogenesis, and increase in lethal factor production from opportunistic infectious agents (e.g., *Pseudomonas aeruginosa*). Formulations are useful

for administration to patients receiving short term treatment with opioids (e.g., patients suffering from post-operative gastrointestinal dysfunction receiving short term opioid administration). Formulations are also useful for administration to subjects receiving chronic opioid administration (e.g., terminally ill patients receiving opioid therapy such as an AIDS patient, a cancer patient, a cardiovascular patient; subjects receiving chronic opioid therapy for pain management; subjects receiving opioid therapy for maintenance of opioid withdrawal).

[0020] Further uses of provided formulations include, for example, prevention, delay, treatment or reduction of severity of symptoms associated with disorders or conditions resulting from normal or aberrant activity of endogenous opioids. Such disorders or condition include, among others, ileus (e.g., post-partum ileus, paralytic ileus), gastrointestinal dysfunction that develop following abdominal surgery (e.g., colectomy, including but not limited to, right hemicolectomy, left hemicolectomy, transverse hemicolectomy, colectomy takedown, and low anterior resection) such as post-operative ileus, and idiopathic constipation. Provided formulations are also useful in treatment of conditions including cancers involving angiogenesis, immune suppression, sickle cell anemia, vascular wounds, retinopathy, and treatment of inflammation associated disorders (e.g., irritable bowel syndrome), immune suppression, and chronic inflammation.

Definitions

[0021] The term "dose-concentrate" refers to a pharmaceutical composition comprising a provided formulation, wherein the concentration of active agent(s) is higher than a typical unit dosage form concentration administered directly to a subject. A dose-concentrate may be used as provided for administration to a subject, but is generally further diluted to a typical unit dosage form concentration in preparation for administration to a subject. The entire volume of a dose-concentrate, or aliquots thereof, may be used in preparing unit dosage form(s) for treatment, for example, by the methods provided herein. In some embodiments, a dose-concentrate is about 2 fold, about 5-fold, about 10-fold, about 25-fold, about 50-fold, about 100-fold, or about 200-fold more concentrated than a unit dosage form. In certain embodiments, a dose concentrate is about 50-fold, about 100-fold, or about 200-fold more concentrated than a unit dosage form.

[0022] As used herein, an "effective amount" of a compound or pharmaceutically acceptable formulation can achieve a desired therapeutic and/or prophylactic effect. In some

embodiments, an "effective amount" is at least a minimal amount of a compound, or formulation containing a compound, which is sufficient for treating one or more symptoms of a disorder or condition associated with modulation of peripheral μ opioid receptors, such as side effects associated with opioid analgesic therapy (e.g., gastrointestinal dysfunction (e.g., dysmotility constipation, etc.), nausea, emesis,(e.g., vomiting), etc.). In certain embodiments, an "effective amount" of a compound, or formulation containing a compound, is sufficient for treating symptoms associated with, a disease associated with aberrant endogenous peripheral opioid or μ opioid receptor activity (e.g., idiopathic constipation, ileus, etc.).

[0023] The term "formulation" refers to a composition that includes at least one pharmaceutically active compound (e.g., at least methylnaltrexone) in combination with one or more excipients or other pharmaceutical additives for administration to a subject. In general, particular excipients and/or other pharmaceutical additives are typically selected with the aim of enabling a desired stability, release, distribution and/or activity of active compound(s) for applications.

[0024] The term "subject", as used herein, means a mammal to whom a formulation or composition comprising a formulation is administered, and includes human and animal subjects, such as domestic animals (e.g., horses, dogs, cats, etc.).

[0025] "Therapeutically active compound" or "active compound" refers to a substance, including a biologically active substance, that is useful for therapy (e.g., human therapy, veterinary therapy), including prophylactic and/or therapeutic treatment. Therapeutically active compounds can be organic molecules that are drug compounds, peptides, proteins, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, nucleoprotein, mucoprotein, lipoprotein, synthetic polypeptide or protein, small molecules linked to a protein, glycoprotein, steroid, nucleic acid, DNA, RNA, nucleotide, nucleoside, oligonucleotides, antisense oligonucleotides, lipid, hormone, and vitamin. Alternatively or additionally, therapeutically active compounds can be any substance used as a medicine for treatment, prevention, delay, reduction or amelioration of a disease, condition, or disorder. Among therapeutically active compounds useful in the formulations of the present invention are opioid antagonist compounds, opioid analgesic compounds, and the like. Further detailed description of compounds useful as therapeutically active compounds is provided below. A therapeutically active compound includes a compound that increases the effect or effectiveness of a second compound, for

example, by enhancing potency or reducing adverse effects of a second compound. The terms "treat" or "treating," as used herein, refers to partially or completely alleviating, inhibiting, delaying onset of, reducing the incidence of, ameliorating and/or relieving a disorder or condition, or one or more symptoms of the disorder, disease or condition.

[0026] The expression "unit dosage" as used herein refers to a physically discrete unit of a formulation appropriate for a subject to be treated. It will be understood, however, that the total daily usage of a formulation of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular subject or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active compound employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active compound employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts.

[0027] The expression "dosage form" refers to means by which a formulation is stored and/or administered to a subject. For example, the formulation may be stored in a vial or syringe. The formulation may also be stored in a container which protects the formulation from light (e.g., UV light). Alternatively a container or vial which itself is not necessarily protective from light may be stored in a secondary storage container (e.g., an outer box, bag, etc.) which protects the formulation from light.

[0028] The present invention provides formulations and dosage forms for parenteral administration of methylnaltrexone, including pharmaceutically acceptable salts thereof. As used herein, "methylnaltrexone" includes N-methylnaltrexone and salts thereof. Methylnaltrexone is described for example in United States patents 4,176,186; 4,719,215; 4,861,781; 5,102,887; 5,972,954; 6,274,591; United States published patent application numbers 20020028825 and 20030022909; and PCT publications WO99/22737 and WO98/25613; the contents of each of which are hereby incorporated by reference.

[0029] In general, pharmaceutically acceptable salts include, but are not limited to, chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, pantothenate, bitartrate, carbonate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate,

carboxylate, benzoate, glutamate, sulfonate, methanesulfonate, ethanesulfonate, benzensulfonate, p-toluenesulfonate, selenate, and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts of compounds. In some embodiments, salts of use in formulations of the invention are those that have been described for methylnaltrexone, e.g., methylnaltrexone bromide, etc. However, the invention is not limited to these specific salts. Other salts and mixtures thereof can be adapted and used in a dose formulation according to the invention so as to achieve the appropriate compound delivery profiles of the invention (e.g., chloride, sulfate, bisulfate, tartrate, nitrate, citrate, bitartrate, phosphate, malate, maleate, bromide, iodide, fumarate, sulfonate, carboxylate, or succinate salts, etc.). Alternatively or additionally, peripheral opioid receptor antagonist (e.g., methylnaltrexone) base, chemical and chiral derivatives thereof and other salts can be used, as appropriate.

[0030] The bromide salt of methylnaltrexone is also referred to, for example, N-methylnaltrexone bromide, N-methylnaltrexone hydrobromide, methylnaltrexone bromide, methylnaltrexone hydrobromide, naltrexone methobromide, N-methylnaltrexone, MNTX, SC-37359, MRZ-2663-BR, and N-cyclopropylmethylnoroxy-morphine-metho-bromide. Methylnaltrexone is available in a powder form from Mallinckrodt Pharmaceuticals, St. Louis, Mo., provided as a white crystalline powder freely soluble in water. Its melting point is 254-256°C. In some embodiments, the invention provides formulations in a vial. In certain embodiments, a formulation is provided in a vial containing a unit dosage of methylnaltrexone. In such embodiments, a formulation may comprise about 0.5 mg to about 200 mg methylnaltrexone bromide. In some embodiments, a unit dosage can contain from about 1 mg to about 80 mg, from about 5 mg to about 40 mg, or from about 8 mg to 12 mg to about 18 mg to about 24 mg.

[0031] Methylnaltrexone has chiral centers and can therefore occur as stereochemical isomers by virtue of the substituent placement on those chiral centers. Such stereochemical isomers are within the scope of the compounds contemplated for use in the present formulations. In the compositions and methods of the present invention, compounds employed may be individual stereoisomers, as well as mixtures of stereoisomers. In certain aspects, methods of the present invention utilize compounds which are substantially pure stereoisomers. All tautomers are also intended to be encompassed within the compositions of the present invention.

[0032] The terms "R" and "S" are used herein, as commonly used in organic chemistry nomenclature, to denote specific configuration of a chiral center. The term "R" refers to "right" and is used to designate the configuration of a chiral center with a clockwise relationship of group priorities (highest to second lowest) when viewed along the bond toward the lowest priority group. The term "S" or "left" is used to designate the configuration of a chiral center with a counterclockwise relationship of group priorities (highest to second lowest) when viewed along the bond toward the lowest priority group. The priority of groups is based upon their atomic number (heaviest isotope first). A partial list of priorities and a discussion of stereochemistry is contained in the book: The Vocabulary of Organic Chemistry, Orchin, et al., John Wiley and Sons Inc., page 126 (1980), which is incorporated herein by reference in its entirety.

[0033] In some embodiments, isolated R-N isomers of methylnaltrexone may be utilized in formulations and methods. As used herein, the designation of "R-N-isomer" of methylnaltrexone refers to such compounds in the (R) configuration with respect to the nitrogen. Isolated isomer compounds include, but are not limited to, R-N isomer methylnaltrexone compounds described in U.S. Patent application serial number 11/441,395 filed May 25, 2006, published WO2006/127899, which is hereby incorporated herein by reference. In some embodiments, the active compound is an R-N isomer methylnaltrexone, or a salt thereof. The R-N isomer of methylnaltrexone has been found in USSN 11/441,395 to be an opioid antagonist.

[0034] In some embodiments, isolated S-N isomers of methylnaltrexone may be utilized in formulations and methods. As used herein, the designation of "S-N-isomer" of methylnaltrexone refers to such compounds in the (S) configuration with respect to the nitrogen. Isolated isomer compounds include, but are not limited to, S-N isomer of methylnaltrexone compounds described in U.S. Patent application serial number 11/441,452, filed May 25, 2006, published WO2006/127898, which is hereby incorporated by reference. In some embodiments, the active compound is an S-N isomer methylnaltrexone, or a salt thereof. The S-N isomer of methylnaltrexone has been found in USSN 11/441,452 to be an opioid agonist.

[0035] In certain embodiments, the methylnaltrexone of formulations described herein is a mixture of stereoisomers characterized in that it has an opioid antagonist effect. For example, the methylnaltrexone may be a mixture of R-N and S-N methylnaltrexone such that a mixture itself has an antagonist effect and would be useful for methods of use described herein for opioid

antagonists. In certain embodiments, R-N methylnaltrexone is used which is substantially free of S-N methylnaltrexone.

[0036] In certain embodiments of the present invention, at least about 99.6%, 99.7%, 99.8%, 99.85%, 99.9%, or 99.95% of methylnaltrexone is in the (R) configuration with respect to nitrogen. Methods for determining the amount of (R)-N-isomer, present in a sample as compared to the amount of (S)-N-isomer present in that same sample, are described in detail in WO2006/127899, the entirety of which is hereby incorporated herein by reference. In other embodiments, methylnaltrexone contains 0.15%, 0.10%, or less (S)-N-isomer.

[0037] The exact amount of methylnaltrexone (or combination of methylnaltrexone and any other particular active agent) that is required to achieve a pharmaceutically effective amount will vary from subject to subject, depending on species, age, and general condition of a subject, severity of the side effects or disorder, identity of the particular compound(s), mode of administration, and the like. A total daily dosage of methylnaltrexone (e.g., methylnaltrexone bromide) will typically be in the range 10-200 mg, preferably 20-100 mg for a 70 kg adult human. A unit dosage formulation according to the invention will usually contain 1-250 mg of active compound (e.g., methylnaltrexone bromide) per unit, 5-100 mg of active compound per unit, 10-50 mg of active compound per unit, or about 8 mg or about 12 mg or about 24 mg of active compound per unit. In certain embodiments, an effective amount of a methylnaltrexone for administration to a 70 kg adult human may comprise about 10 mg to about 50 mg of compound (e.g., methylnaltrexone bromide) per unit dosage, to be administered one or more times a day. It will be appreciated that dose ranges set out above provide guidance for the administration of active compound to an adult. The amount to be administered to for example, an infant or a baby can be determined by a medical practitioner or person skilled in the art and can be lower or the same as that administered to an adult.

Formulations

[0038] The present invention provides formulations that are capable of maintenance of integrity of methylnaltrexone without substantial production of degradants following storage, including storage at room temperature. Thus, the provided formulations are capable of conferring improved storage stability characteristics of delivered methylnaltrexone. For example, in some embodiments, a formulation comprises methylnaltrexone, a calcium salt chelating agent,

an isotonic agent, and a carrier. In some embodiments, a formulation comprises methylnaltrexone, a calcium salt chelating agent, an isotonic agent, a stabilizing agent, and a carrier. In some embodiments, the pH of the formulation is between about a pH of 2 to about a pH of 5.

[0039] The present invention provides formulations and methods for delivery of methylnaltrexone for improved storage and maintenance of pharmaceutical compositions. In particular, the present invention provides formulations that are stable formulations for parenteral administration of methylnaltrexone compositions. Formulations provided for parenteral administration may include sterile solution for injection, sterile suspension for injection, sterile emulsions, and dispersions.

[0040] For example, in some embodiments, formulations comprise methylnaltrexone, and a calcium salt-chelating agent in an isotonic solution. In some embodiments, fomulations comprise methylnaltrexone, a calcium salt chelating agent, and a stabilizing agent in an isotonic solution.

[0041] Generally, provided formulations will include one or more active compound(s) together with one or more excipients, such as, for example, one or more chelating agents, a calcium ion, isotonic agents, carriers, buffers, co-solvents, diluents, preservatives, and/or surfactants, or combinations thereof. One skilled in the art will readily appreciate that the same ingredient can sometimes perform more than one function, or can perform different functions in the context of different formulations, and/or portions of a formulation, depending upon the amount of the ingredient and/or the presence of other ingredients and/or active compound(s). Active compound may comprise about 0.5 mg to about 200 mg methylnaltrexone bromide. In some embodiments, active compound may comprise about 1 mg to about 80 mg, from about 5 mg to about 40 mg, or about 8, or about 12 mg, about 16 mg, about 18 mg, or about 24 mg methylnaltrexone bromide.

[0042] In some embodiments, the formulation comprises a chelating agent. In some embodiments, a chelating agent may be present in an amount from about 0.01 mg/mL to about 2 mg/mL or about 0.1 mg/mL to about 1 mg/mL in the formulation, or about 0.2 mg/mL to about 0.8 mg/mL of the formulation. In some embodiments, a chelating agent may be present in an amount from about 0.2 mg/mL, about 0.3 mg/mL, about 0.4 mg/mL, about 0.5 mg/mL, or about 0.6 mg/mL, in the formulation.

[0043] We have found use of a chelating agent is effective as inhibiting at least one degradant formation. Thus, addition of at least one chelating agent is particularly useful in formulations that include methylnaltrexone, and provides protection from metal-catalyzed degradant production, and/or from precipitation. Appropriate chelating agents include any pharmaceutically acceptable chelating agents and salts thereof. Examples of chelating agents include, but are not limited to ethylenediaminetetraacetic acid (also synonymous with EDTA, edetic acid, versene acid, and sequestrene), and EDTA derivatives, such as sodium EDTA, and potassium EDTA, diammonium EDTA, dipotassium EDTA, disodium EDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium EDTA, HEDTA, and trisodium HEDTA, and related salts thereof. Other chelating agents include niacinamide and derivatives thereof and sodium desoxycholate and derivatives thereof, ethylene glycol-bis-(2-aminoethyl)-N,N,N', N'-tetraacetic acid (EGTA) and derivatives thereof, diethylenetriaminepentaacetic acid (DTPA) and derivatives thereof, N,N-bis(carboxymethyl)glycine (NTA) and derivatives thereof, nitrilotriacetic acid and derivatives thereof. Still other chelating agents include citric acid and derivatives thereof. Citric acid also is known as citric acid monohydrate. Derivatives of citric acid include anhydrous citric acid and trisodiumcitrate-dihydrate. In some embodiments, chelating agent is selected from EDTA or an EDTA derivative or EGTA or an EGTA derivative. In some embodiments chelating agent is EDTA disodium such as, for example, EDTA disodium hydrate.

[0044] In some embodiments, a provided formulation comprises a calcium salt. In some embodiments, a calcium salt may be present in an amount from about 0.01 mg/mL to about 2 mg/mL or about 0.1 mg/mL to about 1 mg/mL in the formulation, or about 0.2 mg/mL to about 0.8 mg/mL of the formulation. In some embodiments, a calcium salt may be present in an amount from about 0.2 mg/mL, about 0.3 mg/mL, about 0.4 mg/mL, about 0.5 mg/mL, or about 0.6 mg/mL, in the formulation.

[0045] We have found the presence of a calcium ion is effective as inhibiting formation of at least one degradant. Thus, addition of at least one calcium salt is particularly useful in formulations that include methylnaltrexone, and provides protection from metal-catalyzed degradant production, and/or from precipitation. Appropriate calcium salts include any pharmaceutically acceptable calcium salts. Examplary of calcium salts include, but are not limited to calcium chloride, calcium acetate, calcium citrate, calcium sulfate, etc.

[0046] In some embodiments, a formulation comprises a calcium ion and a chelating agent included as a single component of the formulation. Thus in some embodiments a calcium salt chelating agent may be present in an amount from about 0.01 mg/mL to about 2 mg/mL or about 0.1 mg/mL to about 1 mg/mL in the formulation, or about 0.2 mg/mL to about 0.8 mg/mL of the formulation. In some embodiments, calcium salt chelating agent may be present in an amount from about 0.2 mg/mL, about 0.3 mg/mL, about 0.4 mg/mL, about 0.5 mg/mL, or about 0.6 mg/mL, in the formulation.

[0047] We have found use of a calcium salt chelating agent is particularly effective as inhibiting formation of at least one degradant. Thus, addition of at least one calcium salt chelating agent is particularly useful in formulations that include methylnaltrexone, and provides protection from metal-catalyzed production of 2,2' bis-methylnaltrexone, and 7-dihydroxy methylnaltrexone, and/or from precipitation. In some embodiments, the formulation comprises a calcium salt chelating agent.

[0048] Appropriate calcium salt chelating agents include any pharmaceutically acceptable chelating agents and calcium salts thereof. Common calcium salt chelating agents include, but are not limited to calcium ethylenediaminetetra acetic acid (EDTA) and calcium salt EDTA derivatives, calcium ethylene glycol-bis-(2-aminoethyl)-N,N,N', N'-tetraacetic acid (EGTA) and calcium salt EGTA derivatives, calcium diethylenetriaminepentaacetic acid (DTPA) and calcium salt DTPA derivatives, calcium N,N-bis(carboxymethyl)glycine (NTA) and calcium salt NTA derivatives, and calcium citrate and derivatives thereof. In some embodiments, chelating agent is selected from calcium EDTA or a calcium salt EDTA derivative or calcium EGTA or a calcium salt EGTA derivative. In some embodiments chelating agent is calcium EDTA disodium such as, for example, calcium EDTA disodium hydrate.

[0049] In some embodiments, a provided formulation comprises at least methylnaltrexone, a calcium salt chelating agent and an isotonic agent. An isotonic agent useful in the present formulations can be any pharmaceutically acceptable isotonic agent. Common isotonic agents include agents selected from the group consisting of sodium chloride, mannitol, lactose, dextrose (hydrous or anhydrous), sucrose, glycerol, and sorbitol, and solutions of the foregoing. In certain embodiments, the formulation comprises methylnaltrexone, an isotonic agent which is sodium chloride, and a calcium salt chelating agent which is calcium EDTA or a calcium salt EDTA derivative. In some embodiments, the EDTA is calcium EDTA disodium.

[0050] In some embodiments, the formulation comprises at least methylnaltrexone, an isotonic agent, a calcium salt chelating agent and a carrier vehicle. In certain embodiments, the carrier vehicle is an aqueous carrier. Aqueous carrier vehicles are known in the art, and include, but are not limited to sterile water, water for injection, sodium chloride, Ringer's injection, isotonic dextrose injection, dextrose and lactated Ringers injection. In some embodiments, the formulation comprises water for injection. In some embodiments, formulations comprise methylnaltrexone or a pharmaceutically acceptable salt thereof, calcium EDTA or a calcium salt EDTA derivative, water for injection, and sodium chloride in an amount such that the final solution is isotonic (e.g., 0.1%, 0.25%, 0.45% 0.65%, 0.9% sodium chloride). In some embodiments, the sodium chloride is present in an isotonic amount, such that final concentration of sodium chloride is 0.65%.

[0051] Still additional components such as stabilizing agents, buffers, co-solvents, diluents, preservatives, and/or surfactants, etc. may be included in provided formulations. In some embodiments, formulations may contain such additional agents which comprise from about 1% to about 30% or about 1% to about 12% of the formulation or about 1% to about 10%, based upon total weight of the formulation. In some embodiments, additional agents may comprise from about 1%, about 2%, about 5%, about 8% or about 10% of the formulation, based upon total weight of the formulation. Optionally included additional ingredients are described below.

[0052] In some embodiments, provided formulations comprise a stabilizing agent. In some embodiments, stabilizing agent may be present in an amount from about 0.01 mg/mL to about 2 mg/mL or about 0.05 mg/mL to about 1 mg/mL in the formulation, or about 0.1 mg/mL to about 0.8 mg/mL in the formulation. In some embodiments, stabilizing agent may be present in an amount from about 0.15 mg/mL, about 0.2 mg/mL, about 0.25 mg/mL, about 0.3 mg/mL, about 0.35 mg/mL, or about 0.4 mg/mL.

[0053] Suitable stabilizing agents for use in formulations of the invention include, but are not limited to glycine, benzoic acid, citric, glycolic, lactic, malic, and maleic acid. In some embodiments, the formulation comprises glycine. In some embodiments, glycine comprises glycine-HCl. In some embodiments, formulations comprise methylnaltrexone, calcium EDTA or a calcium salt EDTA derivative, water for injection, sodium chloride in an amount such that the final concentration is 6.5mg/mL isotonic sodium chloride, and glycine such as glycine HCl.

[0054] In certain embodiments, a stabilizing agent is added to the formulation in an amount sufficient to adjust and maintain the pH of the formulation. Thus, in some embodiments, a stabilizing agent acts as a buffer function in addition to its role as a stabilizer. In some embodiments, a stabilizing agent may act as a buffer agent, so as to maintain the pH of the formulation. In certain embodiments, the pH is between about pH 2.0 and about pH 6.0. In some embodiments, the pH of the formulation is between about pH 2.6 and about pH 5.0. In some embodiments, the pH of the formulation is between about pH 3.0 and about pH 4.0. In some embodiments, the pH of the formulation is between about pH 3.4 and about pH 3.6. In some embodiments, the pH of the formulation is about pH 3.5.

[0055] In some embodiments, provided formulations comprise methylnaltrexone, calcium EDTA or a calcium salt EDTA derivative, water for injection, sodium chloride in an amount such that the final concentration is 6.5mg/mL isotonic sodium chloride, glycine, and the pH of the formulation is between about pH 3.0 and about pH 4.0. In some embodiments, formulations comprise methylnaltrexone or a pharmaceutically acceptable salt thereof, calcium EDTA or a calcium salt EDTA derivative, water for injection, sodium chloride in an amount such that the final concentration is 6.5mg/mL isotonic sodium chloride, glycine, and the pH of the formulation is between about pH 3.4 and about pH 3.6. In some embodiments, formulations comprise methylnaltrexone bromide, calcium EDTA or a calcium salt EDTA derivative, water for injection, sodium chloride in an amount such that the final concentration is 6.5mg/mL isotonic sodium chloride, and glycine, and the formulation has a pH of about 3.5. In certain embodiments, the pH is adjusted with glycine. In some embodiments, glycine is glycine HCl.

[0056] In some embodiments, provided formulations comprise methylnaltrexone bromide, calcium EDTA, water for injection, isotonic sodium chloride, glycine HCl, and the formulation has a pH between about 3.4 and about 3.6. In some embodiments, provided formulations comprise methylnaltrexone bromide at a concentration about 20mg/mL, calcium EDTA at a concentration about 0.4mg/mL, sodium chloride in an amount such that the final concentration is 6.5mg/mL isotonic sodium chloride, and glycine HCl at a concentration about 0.3mg/mL, and the formulation has a pH of about 3.5. In some embodiments, formulations comprise methylnaltrexone bromide at a concentration about 10 mg/mL, calcium EDTA at a concentration about 0.2 mg/mL, sodium chloride in an amount such that the final concentration

is 3.25 mg/mL isotonic sodium chloride, and glycine HCl at a concentration about 0.15 mg/mL, and the formulation has a pH of about 3.5.

[0057] One of ordinary skill in the art will recognize that additional pH adjustments may be required to ensure that a provided formulation has desired pH. Thus, in certain embodiments, further pH adjustment is performed with hydrochloric acid and/or sodium hydroxide.

Additional components

[0058] In some embodiments, formulations may comprise one or more additional agents for modification and/or optimization of release and/or absorption characteristics. For example, as mentioned above, incorporation of buffers, co-solvents, diluents, preservatives, and/or surfactants may facilitate dissolution, absorption, stability, and/or improved activity of active compound(s), and may be utilized in formulations of the invention. In some embodiments, where additional agents are included in a formulation, the amount of additional agents in the formulation may optionally include: buffers about 10% to about 90%, co-solvents about 1% to about 50%, diluents about 1% to about 10%, preservative agents about 0.1% to about 8%, and/or surfactants about 1% to about 30%, based upon total weight of the formulation, as applicable.

[0059] Suitable co-solvents (i.e., water miscible solvents) are known in the art. For example, suitable co-solvents include, but are not limited to ethyl alcohol, propylene glycol.

[0060] Physiologically acceptable diluents may optionally be added to improve product characteristics. Physiologically acceptable diluents are known in the art and include, but are not limited to, sugars, inorganic salts and amino acids, and solutions of any of the foregoing. Representative examples of acceptable diluents include dextrose, mannitol, lactose, and sucrose, sodium chloride, sodium phosphate, and calcium chloride, arginine, tyrosine, and leucine, and the like, and aqueous solutions thereof.

[0061] Suitable preservatives are known in the art, and include, for example, benzyl alcohol, methyl paraben, propyl paraben, sodium salts of methyl paraben, thimerosal, chlorobutanol, phenol. Suitable preservatives include but are not limited to: chlorobutanol (0.3-0.9% W/V), parabens (0.01-5.0% W/V), thimerosal (0.004-0.2% W/V), benzyl alcohol (0.5-5% W/V), phenol (0.1-1.0% W/V), and the like.

[0062] Suitable surfactants are also known in the art and include, e.g., poloxamer, polyoxyethylene ethers, polyoxyethylene sorbitan fatty acid esters polyoxyethylene fatty acid

esters, polyethylene glycol fatty acid esters, polyoxyethylene hydrogenated castor oil, polyoxyethylene alkyl ether, polysorbates, cetyl alcohol, glycerol fatty acid esters (e.g., triacetin, glycerol monostearate, and the like), polyoxymethylene stearate, sodium lauryl sulfate, sorbitan fatty acid esters, sucrose fatty acid esters, benzalkonium chloride, polyethoxylated castor oil, and docusate sodium, and the like, and combinations thereof. In some embodiments the formulation may further comprise a surfactant.

Dosage Forms

[0063] As indicated, the present invention provides dosage forms including unit dosage forms, dose-concentrates, etc. for parenteral administration. Parenteral administration of provided formulations may include any of intravenous injection, intravenous infusion, intradermal, intralesional, intramuscular, subcutaneous injection or depot administration of a unit dose. A unit dosage may or may not constitute a single "dose" of active compound(s), as a prescribing doctor may choose to administer more than one, less than one, or precisely one unit dosage in each dose (i.e., each instance of administration). For example, unit dosages may be administered once, less than once, or more than once a day, for example, once a week, once every other day (QOD), once a day, or 2, 3 or 4 times a day, more preferably 1 or 2 times per day.

[0064] In certain embodiments, a provided dosage form is administered to a rehab patient (patients undergoing rehabilitation for orthopaedic surgery, e.g. joint replacement) every other day or every day. In other embodiments, provided dosage is 12 mg methylnaltrexone.

[0065] In certain embodiments, a provided dosage form is administered to a chronic pain patient every other day or every day. In some embodiments, the pain is malignant or nonmalignant. In other embodiments, provided dosage is 12 mg methylnaltrexone.

[0066] The present invention provides variety of different dosage forms useful for parenteral administration, including, for example, a methylnaltrexone formulation provided in a container (e.g., a vial, ampoule, syringe, bag, dispenser, etc).

[0067] In one embodiment, the formulation is in a vial filled with methylnaltrexone solution, where the solution comprises at least one active compound which is methylnaltrexone, and a calcium salt chelating agent, in an isotonic solution. In one embodiment, a provided formulation is in a vial where the vial is filled with a provided formulation, as described above

and herein. In some embodiments, provided formulation is in a vial from about 1mL capacity to about 50mL capacity. In some embodiments, a vial is about 1mL, about 2mL, about 5mL, about 10mL, about 25mL or about 50mL capacity.

[0068] In one embodiment, a provided formulation is in a syringe or other dispenser filled a provided formulation as described above and herein. In some embodiments, a syringe or dispenser has a capacity from about 1mL to about 20mL. In some embodiments a syringe or dispenser has a capacity of about 1mL, about 2mL, about 2.5mL, about 5mL, about 7.5mL, about 10mL, about 15mL, or about 20mL. In some embodiments, a syringe or dispenser utilizes a hypodermic needle for administration of contents of the syringe or dispenser to a subject. In certain embodiments, a syringe or dispenser utilized a needle-less adapter for transfer of contents of the container to a subject, or, alternatively to a second container for mixing and/or dilution of contents with another solution. A dose-concentrate of a provided formulation can be in a sealed container holding an amount of the pharmaceutical formulation of the invention to be employed over a standard treatment interval such as immediately upon dilution, or up to 24 hours after dilution, as necessary. A solution for intravenous administration can be prepared, for example, by adding a dose-concentrate formulation to a container (e.g., glass or plastic bottles, vials, ampoules) in combination with diluent so as to achieve desired concentration for administration. The amount of dose concentrate added to diluent is a sufficient amount to treat a subject for a period ranging from about 6 hours to about 1 week, but preferably from about 6 or 12 hours to about 24 hours. The container preferably also contains an empty space of sufficient size to permit (i) addition of aqueous solvent plus (ii) additional space as necessary to permit agitation and effect complete mixture of diluted dose concentrate formulation with the added aqueous solvent. A container may be equipped with a penetrable or spikable top, for example, a rubber seal, such that aqueous solvent may be added by penetrating the seal with a hypodermic syringe or other type non-needle based, penetrable seal in order to transfer concentrate contents. In certain embodiments, a provided formulation is provided in a spikable vial. In some embodiments, a provided formulation is provided in a 10 mL spikable vial.

[0069] Addition of aqueous solvent to a liquid dose concentrate may be conveniently used to form unit dosages of liquid pharmaceutical formulations by removing aliquot portions or entire contents of a dose concentrate for dilution. Dose concentrate may be added to an intravenous (IV) container containing a suitable aqueous solvent. Useful solvents are standard

solutions for injection as previously described (e.g., 5% dextrose, saline, lactated ringer's, or sterile water for injection, etc.). Typical unit dosage IV bags are conventional glass or plastic containers having inlet and outlet means and having standard (e.g., 25mL, 50 mL, 100 mL and 150 mL) capacities. Dose concentrate solution of a pharmaceutical formulation of the invention is added to a unit dosage IV container in an amount to achieve a concentration of about 0.1 to about 1.0 mg of methylnaltrexone per mL and preferably from about 0.24 to about 0.48 mg per mL.

[0070] In other embodiments, it may be desirable to package a provided dosage form in a container to protect the formulation from light until usage. In some embodiments, use of such a light-protective container may inhibit one or more degradation pathways. For example, a vial may be a light container which protects contents from being exposed to light. Additionally and/or alternatively, a vial may be packaged in any type of container which protects a formulation from being exposed to light (e.g., secondary packaging of a vial). Similarly, any other type of container may be a light protective container, or packaged within a light protective container.

Preparation of Provided Formulations

[0071] Formulations of the present invention may be prepared in accordance with any of a variety of known techniques, for example as described by M. E. Aulton in "Pharmaceutics: The Science of Dosage Form Design" (1988) (Churchill Livingstone), the relevant disclosures of which are hereby incorporated by reference.

[0072] In one embodiment, a provided formulation is prepared as follows: dry components of a formulation, including active compound (e.g., methylnaltrexone bromide), and calcium salt chelating agent (e.g., calcium EDTA) are dissolved in an appropriate solvent (e.g., an isotonic solution (e.g., isotonic sodium chloride for injection)). Optionally, additional dry and/or wet ingredients (e.g., solvent (e.g., water)), stabilizing agent, or surfactant, may be added. Optionally, additional components, such as stabilizing agents, or surfactants are added to solvent prior to dissolving other components. A provided formulation may be prepared under low oxygen conditions.

[0073] In another embodiment, a provided formulation is prepared as follows: dry components of a formulation, including active compound (e.g., methylnaltrexone bromide), and

calcium salt chelating agent (e.g., calcium EDTA) are dissolved in an appropriate solvent (e.g., an isotonic solution (e.g., isotonic sodium chloride for injection)). Alternatively, dry components of a formulation, including active compound (e.g., methylnaltrexone bromide), and isotonic agent (e.g., sodium chloride) are dissolved in an aqueous solvent (e.g., water for injection) to generate an active compound in an isotonic solution (e.g., methylnaltrexone in isotonic sodium chloride for injection), followed by further addition and dissolution of calcium salt chelating agent (e.g., calcium EDTA) to the solution. Next, the pH of the solution may be adjusted. For example, addition of glycine may adjust the pH to the desired level. For example, addition of glycine HCl may be utilized for addition to the solution to adjust pH to a desired pH (e.g., pH 3-4, pH 3.4-3.6, pH 3.5). Optionally, additional dry and/or wet ingredients (e.g., solvent (e.g., water), stabilizing agent (e.g., glycine), or surfactant, may be added. Optionally, additional components, such as stabilizing agents, surfactants are added to solvent prior to dissolving other components. A provided formulation may be prepared under low oxygen conditions.

[0074] In one embodiment, prepared formulations are incorporated into vials, ampoules, syringes, or dispensers, either alone, or with additional excipients. Typical excipients added to a provided formulation include, but are not limited to surfactants, preservatives, diluents, buffers, co-solvents, etc. Typical amounts of additional excipients added to a solution may include, for example, buffers about 10% to about 90%, co-solvents about 1% to about 50%, diluents about 1% to about 10%, preservative agents about 0.1% to about 8%, and surfactants about 1% to about 30%, based upon total weight.

[0075] A prepared formulation may be subjected to a filtration process in advance of packaging. The filtration process may include, for example in the case of injection preparations, a sterilizing filtration and/or an ultra filtration of the processing solution before packaging to eliminate microorganisms or other contaminating matter from the processing solution.

[0076] A prepared formulation may be subjected to a distributing process to vials (e.g., clear glass vial, amber vials), ampoules, syringes, or dispensers (e.g., auto-dispensers). The distributing process includes, for example in the case of vial packaging, a process distributing a suitable volume of the solution into vials taking the concentration of methylnaltrexone into consideration in order that contained products carry a desired amount of methylnaltrexone.

Isolation and Identification of Degradant Products

[0077] We have identified degradants occurring in methylnaltrexone solutions, as well as certain catalysis routes for formation of degradant(s). Still further, in certain respects, we have identified means to control formation of degradants, thus resulting in lower levels of degradants in liquid formulations containing methylnaltrexone. Provided in further detail in the Example 1 herein are methods and results of such identification, including structures of resulting degradant compounds. Additional Examples further provide characterization of prepared solutions, and identification of mechanisms of catalysis of formation and/or inhibition of formation of degradants.

[0078] Thus, provided are methods for determining the presence of one or more degradants in methylnaltrexone formulations. In certain embodiments, methods of detection of degradants below a designated level are preferred for production of a methylnaltrexone formulation. Detection of individual degradant formation in a methylnaltrexone formulation by HPLC analysis and determining a formulation comprises one or more degradants below a specified level are preferred. In some embodiments the method provides analyzing a methylnaltrexone formulation by HPLC analysis and determining that the level of one or more specified degradants is not exceeded. Preferred concentration levels which are not exceeded for one or more degradants are described in the following paragraphs relating to levels of degradants in provided formulations.

[0079] Further provided are formulations which inhibit formation of methylnaltrexone degradant(s), and confer improved stability characteristics to formulations and compositions and products containing methylnaltrexone formulations. In some embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 2% of methylnaltrexone in the preparation following twelve or eighteen months of storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 1.5% of methylnaltrexone in the preparation following twelve or eighteen months of storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 1% of methylnaltrexone in the preparation following twelve or eighteen months of storage conditions. Preferred storage conditions include room temperature storage.

[0080] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 1.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 1% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0081] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of 2,2' bis-methylnaltrexone degradant product (RRT 1.55) does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration 2,2' bis-methylnaltrexone degradant product (RRT 1.55) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of 2,2' bis-methylnaltrexone degradant product (RRT 1.55) does not exceed about 0.1% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0082] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of 7-dihydroxymethylnaltrexone degradant product (RRT 0.67) does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration 7-dihydroxymethylnaltrexone degradant product (RRT 0.67) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of 7-dihydroxymethylnaltrexone degradant product (RRT 0.67) does not exceed about 0.1% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0083] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of the ring contracted methylnaltrexone degradant product (RRT 0.79) does not

exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration the ring contracted methylnaltrexone degradant product (RRT 0.79) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of the ring contracted methylnaltrexone degradant product (RRT 0.79) does not exceed about 0.1% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0084] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of the aldol dimer methylnaltrexone degradant product (RRT 1.77) does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration the aldol dimer methylnaltrexone degradant product (RRT 1.77) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of the aldol dimer methylnaltrexone degradant product (RRT 1.77) does not exceed about 0.1% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0085] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of the Hoffman elimination methylnaltrexone degradant product (RRT 2.26) does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration the Hoffman elimination methylnaltrexone degradant product (RRT 2.26) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of the Hoffman elimination methylnaltrexone degradant product (RRT 2.26) does not exceed about 0.1% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0086] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of O-methyl methylnaltrexone (RRT 1.66) does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration O-methyl methylnaltrexone (RRT 1.66) does not exceed about 0.25% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of O-methyl methylnaltrexone (RRT 1.66) does not exceed about 0.15% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0087] In some embodiments, methylnaltrexone formulations where the amount of S-N methyl naltrexone in the starting formulation is less than 0.5 wt% (relative to the total amount of methylnaltrexone) are provided wherein the concentration of the S-methylnaltrexone degradant product (RRT 0.89) does not exceed about 0.5% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In some embodiments, methylnaltrexone formulations are provided wherein the concentration the S-methylnaltrexone degradant product (RRT 0.89) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions. In more particular embodiments, methylnaltrexone formulations are provided wherein the concentration of the S-methylnaltrexone degradant product (RRT 0.89) does not exceed about 0.1% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0088] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 1.25% of methylnaltrexone in the preparation following six months of room temperature storage conditions, the concentration 2,2' bis-methylnaltrexone degradant product (RRT 1.55) does not exceed about 0.2% of methylnaltrexone, wherein the concentration 7-dihydroxymethylnaltrexone degradant product (RRT 0.67) does not exceed about 0.2% of methylnaltrexone, the concentration the ring contracted methylnaltrexone degradant product (RRT 0.79) does not exceed about 0.2% of methylnaltrexone, the aldol dimer methylnaltrexone degradant product (RRT 1.77) does not exceed about 0.2% of methylnaltrexone, the Hoffman elimination methylnaltrexone degradant

product (RRT 2.26) does not exceed about 0.2% of methylnaltrexone, and the concentration of O-methyl methylnaltrexone (RRT 1.66) does not exceed about 0.25% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0089] In some embodiments, methylnaltrexone formulations are provided wherein the concentration of total degradation products does not exceed about 0.75% of methylnaltrexone in the preparation following six months of room temperature storage conditions, the concentration of 2,2' bis-methylnaltrexone degradant product (RRT 1.55) does not exceed about 0.1% of methylnaltrexone, wherein the concentration of 7-dihydroxymethylnaltrexone degradant product (RRT 0.67) does not exceed about 0.1% of methylnaltrexone, the concentration of the ring contracted methylnaltrexone degradant product (RRT 0.79) does not exceed about 0.15% of methylnaltrexone, the concentration of aldol dimer methylnaltrexone degradant product (RRT 1.77) does not exceed about 0.05% of methylnaltrexone, the concentration of the Hoffman elimination methylnaltrexone degradant product (RRT 2.26) does not exceed about 0.1% of methylnaltrexone, and the concentration of O-methyl methylnaltrexone (RRT 1.66) does not exceed about 0.15% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

[0090] In other embodiments, methylnaltrexone formulations are provided wherein the concentration of 2,2' bis-methylnaltrexone degradant product (RRT 1.55) does not exceed about 0.2% of methylnaltrexone, wherein the concentration of 7-dihydroxymethylnaltrexone degradant product (RRT 0.67) does not exceed about 0.2% of methylnaltrexone, the concentration of the ring contracted methylnaltrexone degradant product (RRT 0.79) does not exceed about 0.2% of methylnaltrexone, and the concentration of the Hoffman elimination methylnaltrexone degradant product (RRT 2.26) does not exceed about 0.2% of methylnaltrexone in the preparation following six months of room temperature storage conditions.

Combination Products and Combined Administration

[0091] In some embodiments, formulations include one or more other active compounds in addition to methylnaltrexone. In such combination formulations, additional compound(s) may be included in one or more portion(s) that includes methylnaltrexone, may be missing from one or more portions that include methylnaltrexone, and/or may be included in one or more portions that does not include methylnaltrexone. Specifically, the invention encompasses formulations

that deliver at least methylnaltrexone and at least one other active compound. Additionally, the invention encompasses formulations that deliver at least two independent portions of methylnaltrexone, and that further deliver at least one other active compound(s).

[0092] In some embodiments, formulations comprise both an opioid and methylnaltrexone (e.g., a μ opioid receptor antagonist). Such combination products, containing both an opioid and an opioid antagonist, would allow simultaneous relief of pain and minimization of opioid-associated side effects (e.g., gastrointestinal effects (e.g., delayed gastric emptying, altered GI tract motility), etc.).

[0093] Opioids useful in treatment of analgesia are known in the art. For example, opioid compounds include, but are not limited to, alfentanil, anileridine, asimadoline, bremazocine, burprenorphine, butorphanol, codeine, dezocine, diacetylmorphine (heroin), dihydrocodeine, diphenoxylate, ethylmorphine, fedotozine, fentanyl, funaltrexamine, hydrocodone, hydromorphone, levallorphan, levomethadyl acetate, levorphanol, loperamide, meperidine (pethidine), methadone, morphine, morphine-6-glucoronide, nalbuphine, nalorphine, nicomorphine, opium, oxycodone, oxymorphone, papaveretum, pentazocine, propiram, propoxyphene, remifentanyl, sufentanil, tilidine, trimebutine, and tramadol. In some embodiments the opioid is at least one opioid selected from alfentanil, buprenorphine, butorphanol, codeine, dezocine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, nicomorphine, oxycodone, oxymorphone, papaveretum, pentazocine, propiram, propoxyphene, sufentanil and/or tramadol. In certain embodiments, the opioid is selected from morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl, tramadol, and mixtures thereof. In a particular embodiment, the opioid is loperamide. In another particular embodiment, the opioid is hydromorphone. In other embodiments, the opioid is a mixed agonist such as butorphanol. In some embodiments, the subjects are administered more than one opioid, for example, morphine and heroin or methadone and heroin.

[0094] The amount of additional active compound(s) present in combination compositions of this invention will typically be no more than the amount that would normally be administered in a composition comprising that active compound as the only therapeutic agent. In certain embodiments, the amount of additional active compound will range from about 50% to

100% of the amount normally present in a composition comprising that compound as the only therapeutic agent.

[0095] In certain embodiments, formulations may also be used in conjunction with and/or in combination with additional active compounds and/or conventional therapies for treatment of gastrointestinal dysfunction to aid in the amelioration of constipation and bowel dysfunction, For example, conventional therapies include, but may not be limited to functional stimulation of the intestinal tract, stool softening agents, laxatives (e.g., diphelymethane laxatives, cathartic laxatives, osmotic laxatives, saline laxatives, etc), bulk forming agents and laxatives, lubricants, intravenous hydration, and nasogastric decompression.

Kits and Uses of Formulations

Uses

[0096] As discussed above, the present invention provides formulations useful in antagonizing undesirable side effects of opioid analgesic therapy (e.g., gastrointestinal effects (e.g., delayed gastric emptying, altered GI tract motility), etc.). Furthermore, formulations of the invention may be used to treat subjects having disease states that are ameliorated by binding μ opioid receptors, or in any treatment wherein temporary suppression of the μ opioid receptor system is desired (e.g., ileus, etc.). In certain embodiments, methods of use of formulations are in human subjects.

[0097] Accordingly, administration of provided formulations may be advantageous for treatment, prevention, amelioration, delay or reduction of side effects of opioid administration, such as, for example, gastrointestinal dysfunction (e.g., inhibition of intestinal mobility, constipation, GI sphincter constriction, nausea, emesis (vomiting), biliary spasm, opioid bowel dysfunction, colic) dysphoria, pruritis, urinary retention, depression of respiration, papillary constriction, cardiovascular effects, chest wall rigidity and cough suppression, depression of stress response, and immune suppression associated with use of narcotic analgesia, etc, or combinations thereof. Use of provided formulations may thus be beneficial from a quality of life standpoint for subjects receiving administration of opioids, as well as to reduce complications arising from chronic constipation, such as hemorrhoids, appetite suppression, mucosal breakdown, sepsis, colon cancer risk, and myocardial infarction.

[0098] In some embodiments, provided formulations are useful for administration to a subject receiving short term opioid administration. In some embodiments, provided formulations are useful for administration to patients suffering from post-operative gastrointestinal dysfunction.

[0099] In other embodiments, provided formulations are also useful for administration to subjects receiving chronic opioid administration (e.g., terminally ill patients receiving opioid therapy such as an AIDS patient, a cancer patient, a cardiovascular patient; subjects receiving chronic opioid therapy for pain management; subjects receiving opioid therapy for maintenance of opioid withdrawal). In some embodiments, the subject is a subject using opioid for chronic pain management. In some embodiments, the subject is a terminally ill patient. In other embodiments the subject is a person receiving opioid withdrawal maintenance therapy.

[00100] Additional uses for formulations described herein may be to treat, reduce, inhibit, or prevent effects of opioid administration including, e.g., aberrant migration or proliferation of endothelial cells (e.g., vascular endothelial cells), increased angiogenesis, and increase in lethal factor production from opportunistic infectious agents (e.g., *Pseudomonas aeruginosa*). Additional advantageous uses of provided formulations include treatment of opioid-induced immune suppression, inhibition of angiogenesis, inhibition of vascular proliferation, treatment of pain, treatment of inflammatory conditions such as inflammatory bowel syndrome, treatment of infectious diseases and diseases of the musculokeletal system such as osteoporosis, arthritis, osteitis, periostitis, myopathies, and treatment of autoimmune diseases.

[00101] In certain embodiments, formulations of the invention may be used in methods for preventing, inhibiting, reducing, delaying, diminishing or treating gastrointestinal dysfunction, including, but not limited to, irritable bowel syndrome, opioid-induced bowel dysfunction, colitis, post-operative, paralytic ileus, or postpartum ileus, nausea and/or vomiting, decreased gastric motility and emptying, inhibition of the stomach, and small and/or large intestinal propulsion, increased amplitude of non-propulsive segmental contractions, constriction of sphincter of Oddi, increased anal sphincter tone, impaired reflex relaxation with rectal distention, diminished gastric, biliary, pancreatic or intestinal secretions, increased absorption of water from bowel contents, gastro-esophageal reflux, gastroparesis, cramping, bloating, abdominal or epigastric pain and discomfort, constipation, idiopathic constipation, post-operative gastrointestinal dysfunction following abdominal surgery (e.g., colectomy (e.g., right

hemicolectomy, left hemicolectomy, transverse hemicolectomy, colectomy takedown, low anterior resection) or hernia repair), and delayed absorption of orally administered medications or nutritive substances.

[00102] Provided formulations are also useful in treatment of conditions including cancers involving angiogenesis, immune suppression, sickle cell anemia, vascular wounds, and retinopathy, treatment of inflammation associated disorders (e.g., irritable bowel syndrome), immune suppression, chronic inflammation.

[00103] In still further embodiments, veterinary applications (e.g., treatment of domestic animals, e.g. horse, dogs, cats, etc.) of use of formulations are provided. Thus, use of provided formulations in veterinary applications analogous to those discussed above for human subjects is contemplated. For example, inhibition of equine gastrointestinal motility, such as colic and constipation, may be fatal to a horse. Resulting pain suffered by the horse with colic can result in a death-inducing shock, while a long-term case of constipation may also cause a horse's death. Treatment of equines with peripheral opioid antagonists has been described, e.g., in U.S. Patent Publication No. 20050124657 published January 20, 2005.

[00104] It will also be appreciated that formulations of the present invention can be employed in combination therapies, that is, methylnaltrexone and compositions thereof, can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. Particular combination therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that therapies employed may achieve a desired effect for the same disorder (for example, a formulation may be administered concurrently with another compound used to treat the same disorder), or they may achieve different effects (e.g., control of any adverse effects). As used herein, additional therapeutic compounds which are normally administered to treat or prevent a particular disease, or condition, are known as "appropriate for the disease, or condition, being treated".

[00105] In other embodiments, provided formulations and dosage forms are useful in preparation of medicaments, including, but not limited to medicaments useful in the treatment of side effects of opioid administration (e.g., gastrointestinal side effects (e.g., inhibition of intestinal motility, GI sphincter constriction, constipation, nausea, emesis) dysphoria, pruritis,

etc.) or a combination thereof. Provided formulations are useful for preparations of medicaments, useful in treatment of patients receiving short term opioid therapy (e.g., patients suffering from post-operative gastrointestinal dysfunction receiving short term opioid administration) or subjects using opioids chronically (e.g., terminally ill patients receiving opioid therapy such as an AIDS patient, a cancer patient, a cardiovascular patient; subjects receiving chronic opioid therapy for pain management; or subjects receiving opioid therapy for maintenance of opioid withdrawal). Still further, preparation of medicaments useful in the treatment of pain, treatment of inflammatory conditions such as inflammatory bowel syndrome, treatment of infectious diseases, treatment of diseases of the musculokeletal system such as osteoporosis, arthritis, osteitis, periostitis, myopathies, treatment of autoimmune diseases and immune suppression, therapy of post-operative gastrointestinal dysfunction following abdominal surgery (e.g., colectomy (e.g., right hemicolectomy, left hemicolectomy, transverse hemicolectomy, colectomy takedown, low anterior resection), idiopathic constipation, and ileus), and treatment of disorders such as cancers involving angiogenesis, chronic inflammation and/or chronic pain, sickle cell anemia, vascular wounds, and retinopathy.

Pharmaceutical Kits and Packaging

[00106] Still further encompassed by the invention are pharmaceutical packs and/or kits. Pharmaceutical packs and/or kits provided may comprise a formulation and a container (e.g., a vial, ampoule, bottle, syringe, and/or dispenser package, or other suitable container). In some embodiments, provided kits may optionally further include a second container comprising a suitable aqueous carrier for dilution of the reconstitute for preparation of administration to a subject via IV administration. In some embodiments, contents of provided formulation container and solvent container combine to form a unit dosage form.

[00107] In some embodiments, a formulation of the invention may be useful in conjunction with patient controlled analgesia (PCA) devices, wherein a patient can administer opioid analgesia as required for pain management. In such instances, co-administration of provided formulations may be useful to prevent adverse side effects of opioid administration. Thus, kits of the invention may comprise a formulation for administration of methylnaltrexone contained within a cartridge for use in conjunction with PCA device.

[00108] In some embodiments, a formulation of the invention may be useful in conjunction with a diluent container suitable for frozen storage, wherein a formulation is diluted in suitable diluent, and provided in a container suitable for freezing. In some embodiments, such frozen containers may be thawed prior to intravenous administration of methylnaltrexone to a subject. Thus, kits of the invention may comprise a formulation for administration of methylnaltrexone in a container suitable for frozen storage, and thawing prior to administration to a subject. In some embodiment, such a container is a frozen intravenous bag.

[00109] Optionally, a single container may comprise one or more compartments for containing lyophilized formulation, and/or appropriate aqueous carrier for dilution. In some embodiments, a single container may be appropriate for modification such that the container may receive a physical modification so as to allow combination of compartments and/or components of individual compartments. For example, a foil or plastic bag may comprise two or more compartments separated by a perforated seal which may be broken so as to allow combination of contents of two individual compartments once the signal to break the seal is generated. A pharmaceutical pack or kit may thus comprise such multi-compartment containers including lyophilized formulation and appropriate solvent for reconstitution and/or appropriate aqueous carrier for dilution of reconstitute. Optionally, instructions for use are additionally provided in such kits.

[00110] In some embodiments, a pharmaceutical kit comprises a formulation in a dilution package or container wherein a needle-less exchange mechanism allows for combination of formulation and with isotonic solution for preparation for intravenous administration. For example, in certain non-limiting examples, a formulation of the invention may be utilized in conjunction with a MINIBAG® Plus diluent container system (Baxter), or an ADD VANTAGE® diluent container (Hospira) system.

[00111] Optionally, instructions for use are additionally provided in such kits of the invention. Such instructions may provide, generally, for example, instructions for dosage and administration. In other embodiments, instructions may further provide additional detail relating to specialized instructions for particular containers and/or systems for administration. Still further, instructions may provide specialized instructions for use in conjunction and/or in combination with additional therapy. In one non-limiting example, the formulations of the invention may be used in conjunction with opioid analgesia administration, which may,

optionally, comprise use of a patient controlled analgesia (PCA) device. Thus, instructions for use of provided formulations may comprise instructions for use in conjunction with PCA administration devices.

[00112] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

EXEMPLIFICATION

PART I: Stability of Provided Formulations

Example 1

Identification and characterization of degradants of methylnaltrexone formulations.

[00113] Previously, at least three degradation products were demonstrated from HPLC analysis in 20 mg/mL isotonic saline solution (identified as RRT peaks at about 0.72, 0.89, and 1.48 when products were analyzed by HPLC). See, e.g., US Patent Application Publication No. 20040266806A1, published December 30, 2004. We examined 20 mg/mL saline methylnaltrexone solutions for production of degradants, and identification of degradants, as well as identification of inhibitors of formation of different degradant products. We have identified and characterized degradants which accumulate in certain methylnaltrexone solutions. In these degradation experiments, and in the formulations prepared in the examples, R-N-methylnaltrexone was used having less than 0.15 weight percent S-N-methylnaltrexone based on the total weight of methylnaltrexone.

[00114] For HPLC analysis, two (2) different methods were utilized to obtain the data set forth herein. These methods are summarized below:

Method A:

Column: Prodigy ODS-3 15cm X 2.0mm, 3μm particles (Phenomenex)
Flow rate: 0.25 mL/min
Detection: UV, 280 nm
Mobile phase: strength: Isocratic: 75:25 (v/v) 0.1% TFA in Water/Methanol
Mobile phase: purity: Gradient as follows:
Solvent A: 95:5 (v/v) 0.1% TFA in Water/Methanol
Solvent B: 35:65 (v/v) 0.1% TFA in Water/Methanol
Sample Solvent: 0.05M Dibasic Sodium Phosphate pH 6.8
Gradient Program:

Time (Min)	% Mobile Phase A
0	100
45	50
45.1	100
60	100

Column Temperature: 50°C

Method B: (purity)

Column: Prodigy ODS-3 15cm X 4.6 mm, 3µm particles (Phenomenex)
Flow rate: 1.5 mL/min
Detection: UV, 280 nm
Mobile phase: Gradient as follows:
Solvent A: 95:5 (v/v) 0.1% TFA in Water/Methanol
Solvent B: 25:75 (v/v) 0.1% TFA in Water/Methanol
Sample Solvent: 0.05M Dibasic Sodium Phosphate pH 6.8
Gradient Program:

Time (Min)	% Mobile Phase A
0	100
45	50
45.1	100
60	100

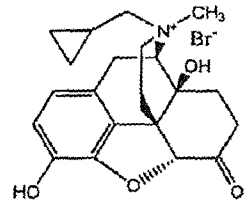
Method B: (strength)

Column: Prodigy ODS-3 15cm X 4.6 mm, 3µm particles (Phenomenex)
Flow rate: 1.0 mL/min
Detection: UV, 280 nm
Mobile phase: Gradient as follows:
Solvent A: 95:5 (v/v) 0.1% TFA in Water/Methanol
Solvent B: 25:75 (v/v) 0.1% TFA in Water/Methanol
Sample Solvent: 0.05M Dibasic Sodium Phosphate pH 6.8
Gradient Program:

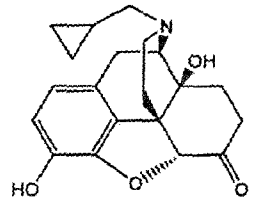
Time (Min)	% Mobile Phase A
0	95
1.0	85
12.0	50
15.0	95
20.0	95

[00115] The following compounds were identified in the stability studies using HPLC analysis (Method A) of samples under the indicated storage conditions, and, unless otherwise noted, had the following associated calculated relative retention times:

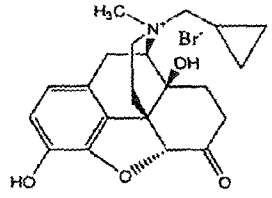
Methylnaltrexone bromide RRT 1.00



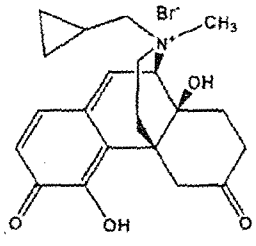
Naltrexone base RRT 1.17



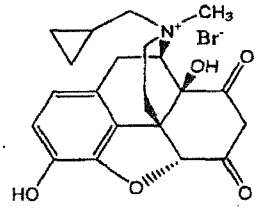
S-Methylnaltrexone RRT 0.86 or 0.89



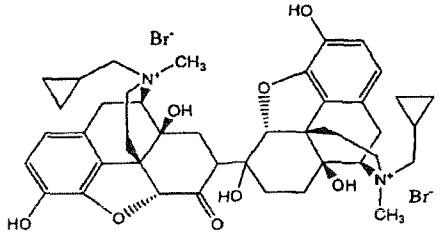
Quinone RRT 0.89 (for Tables 11C-2, 11C-3, 12A-2, 12B-2, 12C-2, 12D-2)



8-ketomethylnaltrexone bromide RRT 0.49

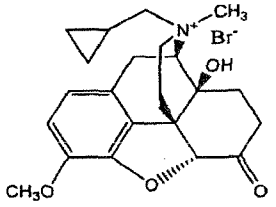


Aldol dimer RRT 1.77

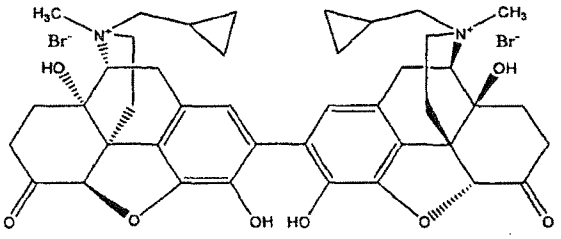


O-Methyl methylnaltrexone RRT 1.66

(3-methyoxy naltrexone methobromide)



2,2,bis-Methylnaltrexone RRT 1.55

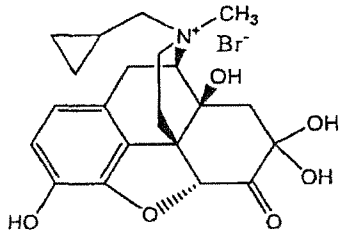


[00116] Naltrexone base, S-methylnaltrexone, and O-methyl methylnaltrexone are each compounds found in initial production samples. Additional impurities/degradants formed and identified in methylnaltrexone formulations include 8-ketomethylnaltrexone bromide (RRT

0.49), the aldol dimer (RRT 1.77), O-methyl methylnaltrexone (RRT 1.66), and the 2,2 bis-methylnaltrexone (RRT 1.55), as well as additional degradants resulting at relative retention time of 0.67, 0.79 and 2.26.

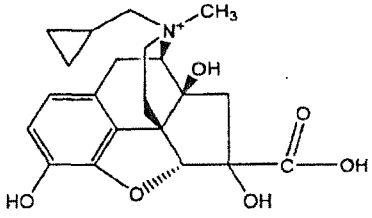
[00117] Each of the three additional degradants were identified by NMR analysis following isolation from column eluates, and further characterized as described herein. The 0.67 degradant has been identified as 7-dihydroxy methylnaltrexone; the 0.79 degradant has been identified as a ring contracted form ((3R,4R,4aS,6aR,11bS)-6-carboxy-3-(cyclopropylmethyl)-4a,6,8-trihydroxy-3-methyl-1,2,3,4,4a,5,6,6a-octahydro-4,11-methano[1]benzofuro[3',2':2,3]cyclopenta[1,2-c]pyridin-3-ium); and the 2.26 degradant has been identified as a Hoffman elimination product (see the following compound names, relative retention times, and associated structure; see also, Figure 4).

7-Dihydroxy methylnaltrexone RRT 0.67

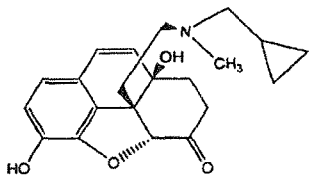


Ring Contraction product RRT 0.79

(3R,4R,4aS,6aR,11bS)-6-carboxy-3-(cyclopropylmethyl)-4a,6,8-trihydroxy-3-methyl-1,2,3,4,4a,5,6,6a-octahydro-4,11-methano[1]benzofuro[3',2':2,3]cyclopenta[1,2-c]pyridin-3-ium)



Hoffman elimination product RRT 2.26



[00118] Results of stability studies in tables set forth in the following examples demonstrate resulting levels of each of the degradants identified in samples using HPLC analysis. Stability test procedures used in the following examples include standard pharmaceutical stability studies according to ICH guidelines, under conditions of 25°C/60% relative humidity, 40°C/65% relative humidity, and/or 70°C. **Figure 4** depicts three of the major resulting degradants, and the associate proposed mechanisms for catalysis of formation and/or methods of inhibition of formation which have been identified and further described in the examples that follow.

[00119] One of ordinary skill in the art will appreciate that minor modifications in an HPLC method or sample preparation can result in a shift of RRT. Thus, it will be appreciated that the RRT values reported herein may shift depending upon actual conditions.

Example 2

Inhibition of metal and calcium mediated degradation of methylnaltrexone formulations.

[00120] Inhibition of metal-catalyzed formation of 2,2'bis methylnaltrexone. We have found $Fe3^{+}$ facilitates degradation of methylnaltrexone bromide in solution, resulting in formation of a 2,2'bis methylnaltrexone degradant. We have found by HPLC analysis (Method B) the 2,2'bis methylnaltrexone degradant results in a peak having an RRT about 1.55. Fe^{3+} is an ion that can get into the liquid formulation from several sources. For example, it can be leached from stainless steel process equipment, syringe needles, stoppers and amber vials. EDTA, as a metal chelating agent sequesters the available Fe^{3+} in the solution, thereby preventing catalysis of the undesirable metal-catalyzed reactions. Methylnaltrexone solutions were prepared in 0.9% NaCl, in the presence of iron and various concentrations of sodium EDTA and calcium EDTA. Used throughout the experiments sodium EDTA is EDTA disodium dihydrate, and the terms sodium EDTA, EDTA disodium dihydrate, and NaEDTA are used interchangeably throughout. Used throughout the experiments calcium EDTA is calcium EDTA

disodium, and the terms calcium EDTA, calcium EDTA disodium, and CaEDTA are used interchangeably throughout. Formation of 2,2'bis methylnaltrexone was assessed at room temperature as well as at 40 °C. Addition of either sodium or calcium EDTA solution was effective at inhibiting formation of the 2,2'bis methylnaltrexone degradant. See **Figure 1A** and **Figure 1B**. Thus, chelating action will facilitate methylnaltrexone bromide stability in solution at room temperature.

[00121] Inhibition of metal-catalyzed formation of 7-dihydroxy-methylnaltrexone. We have found EDTA inhibits metal catalyzed formation of a 7-dihydroxy-methylnaltrexone degradant in methylnaltrexone solution. We have found by HPLC analysis (Method B) the 0.67 peak degradant to be the presence of 7-dihydroxy methylnaltrexone. Methylnaltrexone solutions were prepared in 0.9% NaCl, in the presence of iron and various concentrations of EDTA. Formation of 7-dihydroxy methylnaltrexone was assessed. Addition of either EDTA solution was effective at inhibiting formation of the 7-dihydroxy methylnaltrexone degradant. See Table 1.

TABLE 1: Peak area of RRT 0.67 degradant of 20 mg/ml MNTX at room temperature in presence of 1mm Fe+3

Sample name	Initial	1 hour	2 hours	3 hours	4 hours
MNTX+1mmFe+3	0.7 (0.017%)	0.7 (0.019%)	0.99 (0.024%)	1.16 (0.028%)	1.42 (0.035%)
MNTX+0.25mmEDTA+1mm Fe+3	0	0.72 (0.018%)	0.88 (0.019%)	0.9 (0.022%)	1.2 (0.029%)
MNTX+0.5mmEDTA+1mm Fe+3	0	0.6 (0.015%)	0.87 (0.02%)	0.95 (0.023%)	1.19 (0.029%)
MNTX+0.75mmEDTA+1mm Fe+3	0	0.58 (0.014%)	0.62 (0.013%)	0.75 (0.018%)	0.81 (0.02%)
MNTX+1mmEDTA+1mm Fe+3	0	0.46 (0.011%)	0.57 (0.012%)	0.68 (0.016%)	0.68 (0.016%)

[00122] We have found Ca^{2+} chelating agent provides additional inhibition of formation of a 7-dihydroxy-methylnaltrexone degradant as compared to Na^{2+} chelating agent. Methylnaltrexone solutions were prepared in 0.9% NaCl, in the presence of iron and various concentrations of sodium EDTA and calcium EDTA. Formation of 7-dihydroxy-

methylnaltrexone was assessed at room temperature as well as at 40° C. Addition of calcium EDTA solution was highly effective at inhibiting formation of the 7-dihydroxy-methylnaltrexone degradant at both temperatures. See **Figure 2A** and **Figure 2B**. Use of calcium facilitates methylnaltrexone bromide stability in solution at room temperature. Furthermore, long term storage of solution at either room temperature or 40°C./75% relative humidity also demonstrated stabilization and inhibition of 7-dihydroxy methylnaltrexone degradant formation when calcium EDTA was present. After one month at room temperature, resultant production of 7-dihydroxy-methylnaltrexone was reduced from 0.34% to 0.11% in the presence of calcium EDTA. Furthermore, at 40°C/75% RH, degradant was reduced from 0.64% in saline solution alone to 0.14% in sample containing calcium EDTA. See **Figure 2C** and **Figure 2D**.

[00123] Preparation of an improved room temperature methylnaltrexone forumulation. Our results have shown a methylnaltrexone formulation comprising a saline solution of active compound plus calcium salt-chelating agent results in a formulation having improved room temperature stability characteristics. Preparation of such improved formulations comprise use of the following exemplary components:

Active	Methylnaltrexone bromide	(5 to 40 mgs)
Chelating agent	Calcium EDTA	(0.05 to 1.5 mgs)
Isotonic Delivery Vehicle	0.9% Normal Saline	(1 to 1.25 mL)

[00124] For a 0.6 mL fill or 1.25mL fill, 20 or 30 mgs of methylnaltrexone bromide were dissolved in 0.9% sodium chloride; and 0.24mg or 0.5 mg of calcium EDTA were also dissolved in the solution. Resulting solutions were prepared and filter sterilized at ambient conditions, and resulting formulations filled into clear glass vials, ampoules, syringes or auto-dispensers.

TABLE 2 Formulation

INGREDIENTS	0.6 mL/VIAL	1.25 mL/VIAL
Methylnaltrexone bromide	20 mg	30 mg
Calcium EDTA, NF	0.24 mg	0.5 mg
Sodium Chloride	0.65 %	0.65 %

Example 3

Inhibition of pH dependent degradation of methylnaltrexone formulations

[00125] Inhibition of pH influenced formation of methylnaltrexone degradants. We have found in the presence of Ca^{2+} and EDTA, degradation of methylnaltrexone bromide in solution occurs under some stability conditions, resulting in formation of a third-methylnaltrexone degradant. We have found by HPLC analysis (Method B) the degradant results in a peak having an RRT about 0.79. Identification and production of the 0.79 degradant is described in U.S. provisional patent application 60/835,687, filed August 4, 2006, filed concurrently with the present application, the contents of which are incorporated herein in their entirety by reference.

[00126] Formation of the 0.79 methylnaltrexone degradant was lower at room temperature in the CaEDTA formulation described in Example 2 above as compared to refrigerated methylnaltrexone in saline solution. Methylnaltrexone solution as described in Example 2 containing CaEDTA was compared to a control refrigerated methylnaltrexone solution in saline and formulations assessed for production of 0.79 degradant formation (room temperature CaEDTA 0.03% vs. refrigerated control saline 0.06%). See **Figure 3A** and **Figure 3B**. Use of calcium EDTA appears to facilitate production of the 0.79 degradant under our accelerated stability conditions, however, as it was found at 40°C/75% RH the 0.79 degradant increases from control 0.19% to 0.38% in the presence of CaEDTA. Furthermore, the peak RRT 0.79 degradant increases from 0.03% at room temperature to 0.4% at 40°C/75% RH in 1 month. Thus, while the formulation described above in Example 2 controls degradants RRT 0.67 and RRT 1.55, degradant appearing at RRT 0.79 remains under accelerated stability conditions of 40°C/75% RH.

[00127] We found reduction in pH as well as the presence of glycine resulted in stabilization of the 0.79 degradant. Table 4, summarizes the formulation stability without pH control at 70°C. The formulation has a pH of 5.6. The data confirms that a formulation containing Ca EDTA does limit the formation of 0.67 and RRT 1.55 but does not reduce RRT 0.79. After only a few days RRT 0.79 grows to over 1.0%. Each of the peaks resulting in the HPLC is represented in the table. For those products identified by the peaks: RRT 0.89 represents S-MNTX; RRT 1.17 represents naltrexone base; RRT 1.55 represent 2,2 bis methylnaltrexone; RRT 1.66 represents O-methyl-methylnaltrexone; RRT 1.77 represents aldol

dimer formation; and RRT 2.26 represents Hoffman elimination degradant formation. BRL= below recordable limit.

[00128] We tested whether the 0.79 degradant is pH dependent, and the optimum pH range for a solution. Table 5 summarizes the stability of prepared solutions. Additionally, Table 6 summarizes stability of prepared solutions at 40°C/75% Relative Humidity and at 70°C, with and without pH adjustment with glycine. We found that as additional glycine HCl is added to solution, the amount of degradant at RRT 0.79 formed is greatly reduced and confirms the stability of the formulation with respect to RRT 0.79 is stabilized by the presence of glycine. See Tables 5 and 6.

TABLE 4 -Stability data of MNTX 12 mg/vial, 0.28 mg/vial CaEDTA and 0.65% Sodium Chloride pH(5.6) at 70°C

	Initial (mg)	RRT 0.38	RRT 0.49	RRT 0.67	RRT 0.79	RRT 0.89	RRT 1.17	RRT 1.55	RRT 1.66	RRT 1.77	RRT 1.89	RRT 1.96	RRT 2.01	RRT 2.26	Total
Specifications	NA	0.2	0.5	0.5	0.5	0.15	0.15	0.5	0.15	0.5	0.2	0.2	0.2	0.5	NA
Initial	20 (100)	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
70°C															
Time and Days 3	19.9 (99.5)	BRL	BRL	0.07	1.0	BRL	BRL	BRL	0.13	BRL	BRL	BRL	BRL	1.02	2.22
7	19.7 (98.5)	BRL	BRL	0.09	1.5	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	1.58	3.28

TABLE 5: Stability of MNTX formulation 20 mg/ml , 0.4 mg/ml CaEDTA, 0.65% Sodium Chloride with pH adjusted with Glycine HCl

	Initial (mg)	RRT 0.38	RRT 0.49	RRT 0.67	RRT 0.79	RRT 0.89	RRT 1.17	RRT 1.55	RRT 1.66	RRT 1.77	RRT 1.89	RRT 1.96	RRT 2.01	RRT 2.26	Total
Specifications	NA	0.2	0.5	0.5	0.5	0.15	0.15	0.5	0.15	0.5	0.2	0.2	0.2	0.5	NA
pH 3 at 40°C/75% Relative Humidity															
Time and Days Initial	19.8	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
14	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
21	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
30	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12

Table 5 cont.

pH 3.5 at 40°C/75% Relative Humidity															
Initial	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
7	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
14	20.0	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
21	20.3	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
30	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
pH 4 at 40°C/75% Relative Humidity															
Initial	20.0	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
14	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
21	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.14	0.06	BRL	BRL	BRL	BRL	0.20
30	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	0.06	BRL	BRL	BRL	BRL	0.17

Table 6: Stability of MNTX formulation 20 mg/ml , 0.4 mg/ml CaEDTA, 0.65% Sodium Chloride *with pH adjusted with Glycine HCl*

	Initial (mg)	**RRT 0.38**	**RRT 0.49**	**RRT 0.67**	**RRT 0.79**	**RRT 0.89**	**RRT 1.17**	**RRT 1.55**	**RRT 1.66**	**RRT 1.77**	**RRT 1.89**	**RRT 1.96**	**RRT 2.01**	**RRT 2.26**	**Total**
Specifications	NA	0.2	0.5	0.5	0.5	0.15	0.15	0.5	0.15	0.5	0.2	0.2	0.2	0.5	NA
pH 3 at 70°C															
Time and Days Initial	19.8 (100)	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
10	19.6 (99)	BRL	BRL	0.04	0.04	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	0.06	0.12
14		BRL	BRL	0.07	0.05	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	0.09	0.32
pH 3.5 at 70°C															
Initial	19.9 (100)	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
5	20.2 (101.5)	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.13	BRL	BRL	BRL	BRL	0.08	0.27
7	20.1	BRL	BRL	0.08	0.07	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	0.11	0.38
12	20.2	BRL	BRL	0.06	0.15	BRL	BRL	BRL	0.11	0.06	BRL	BRL	BRL	0.18	0.56

pH 4 at 70^{0}C															
Initial	20.0 (100)	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
10	19.9 (99.5)	BRL	BRL	0.05	0.21	BRL	BRL	BRL	0.13	BRL	BRL	BRL	BRL	0.23	0.39
14		BRL	BRL	0.04	0.27	BRL	BRL	BRL	0.13	BRL	BRL	BRL	BRL	0.28	0.72

Preparation of a pH adjusted, improved room temperature formulation. Listed below, in Table 7 and Table 8, are developed formulations containing glycine HCl, including a pH adjustment step in the process, where the range of pH is 3.4 - 3.6 with a target pH 3.5. While not being bound by theory, this is based on the idea that while pH 3.0 is stable, the amount of irritation and sting at the site of injection would be undesirable. Furthermore, at pH 4.0, RRT 0.79 degradant begins to form. Glycine HCl is commonly used in subcutaneous formulations for pH adjustment, and has less propensity to cause site of injection stinging as results with use of citrate buffer. When glycine HCl is used to adjust the pH of formulations containing methylnaltrexone, controlling degradation is also evident. A solution containing methylnaltrexone including both CaEDTA and 0.3mg/mL glycine HCl where the pH is adjusted to 3.4 – 3.6 will inhibit the formation of RRT 1.55 and greatly reduce the formation of degradants RRT 0.67 and RRT 0.79. A room temperature liquid formulation consisting of methylnaltrexone, CaEDTA, 0.65% NaCl, 0.3mg/mL glycine HCl with a pH to 3.5 may be developed as either a subcutaneous administration or intravenous administration formulation.

[00129] Preparation of such improved formulations comprises use of the following exemplary components:

Active	Methylnaltrexone bromide	(5 to 40 mgs)
Chelating agent	Calcium EDTA	(0.05 to 1.5)
Isotonic Delivery Vehicle	0.65% Normal Saline	(0.5 to 1.75 mL)
Stabilizer	glycine HCl	0.3 mg/mL
		pH 3.4 –3.6

QS to final Volume

TABLE 7 Formulation

INGREDIENTS	12 Mg/VIAL[A]	16 Mg/VIAL[A]	
Methylnaltrexone bromide	12 mg	16 mg	20 mg/mL
Calcium EDTA disodium dihydrate, NF	0.24 mg	0.0.32 mg	0.4 mg/mL
Sodium Chloride	3.9 mg	5.20 mg	6.5 mg/mL
Glycine HCL	0.18 mg	0.0.24 mg	0.3 mg/mL
	pH 3.5	pH 3.5	pH 3.5
Water for Injection, USP	QS to 0.6	QS to 0.8	

[A]3mL West flint glass vial with 13 mm West 4432/50 Fluorotec stopper and West 13 FO CS TE 3769 Blue Cap.

[00130] For example, for preparation of a 12 mg/Vial, 12 mgs of methylnaltrexone bromide and 3.9 mg sodium chloride were dissolved in water for injection; then 0.24 mg of calcium EDTA added and dissolved the final solution brought to a final fill volume of 0.6mL.. The pH was adjusted with Glycine HCl to between 3.4 - 3.6, optimally pH 3.5. Resulting solution was prepared, and filtered through 0.45 and 0.22 micron PVDF filters. Resulting solution was filled into clear glass vials under low oxygen conditions. Any suitable containers, including vials, ampoules, syringes or auto-dispensers may be utilized. Resulting preparations are stored at or below room temperature, without freezing. Resultant formulation may be used for parenteral administration, either for subcutaneous administration, or for intravenous administration applications. See Table 7.

[00131] Similarly, the levels of ingredients may be adapted to a final fill volume of 0.8 (or any other preferred final volume) to obtain the same concentrations. See Table 7.

TABLE 8 Formulation

INGREDIENTS	12 Mg/VIAL[A]	16 Mg/VIAL[A]	
Methylnaltrexone bromide	12 mg	16 mg	10 mg/mL
Calcium EDTA disodium dihydrate, NF	0.24 mg	0.0.32 mg	0.2 mg/mL
Sodium Chloride	3.9 mg	5.20 mg	3.25 mg/mL
Glycine HCL	0.18 mg	0.0.24 mg	0.15 mg/mL
	pH 3.5	pH 3.5	pH 3.5
Water for Injection, USP	QS to 1.2	QS to 1.6	

[A]3mL West flint glass vial with 13 mm West 4432/50 Fluorotec stopper and West 13 FO CS TE 3769 Blue Cap.

[00132] In an alternative exemplary formulation, for a 12 mg/Vial, 12 mgs of methylnaltrexone bromide and 3.9 mg sodium chloride were dissolved in water for injection; then 0.24 mg of calcium EDTA added and dissolved and the final solution brought to a final fill

volume of 1.2 mL. The pH was adjusted with Glycine HCl to between 3.4 - 3.6, optimally pH 3.5. Resulting solution was prepared, and filtered through 0.45 and 0.22 micron PVDF filters. Resulting solution was filled into clear glass vials under low oxygen conditions. Any suitable containers, including vials, ampoules, syringes or auto-dispensers may be utilized. Resulting preparations are stored at or below room temperature, without freezing. Resultant formulation may be used for parenteral administration, either for subcutaneous administration, or for intravenous administration applications. See Table 8.

[00133] Similarly, the levels of ingredients may be adapted to a final fill volume of 1.6 (or any other preferred final volume) to obtain the same concentrations. See Table 8.

Example 4

Comparison and Evaluation of Buffer Compatibility

[00134] Evaluation of phosphate buffers solution stability. We have also assessed different buffers to determine compatibility and whether various conditions would convey further stability to methylnaltrexone solutions. Table 9 and Table 10 show results (HPLC Method A) of total degradant formation over time in methylnaltrexone solutions prepared in phosphate solution (Table 9), and glycine solution (Table 10). We found at pH 7, glycine provides better stability characteristics to samples than phosphate.

Table 9: Stability of MNTX in pH 7, 0.02M Phosphate* Solution

Condition	Elapsed Time	Strength (mg/ml)	% Initial	Total Impurities (% Total Area)	pH of Formulation	Appearance and Description
Room Temperature	0time	0.988	100	0.025	7.09	Clear, colorless solution
	1 day	0.988	100	0.134	7.12	Clear, colorless solution
	2 days	0.996	100.8	0.262	7.11	Clear, colorless solution
	6 days	0.999	101.1	0.786	7.14	Clear, colorless solution
	9 days	0.999	101.1	1.25	7.14	Clear, colorless solution
	14 days	0.988	100.0	1.561	7.14	Clear, colorless solution
	21 days	0.971	98.3	2.07	7.09	Clear, colorless solution

40°C	0time	1.092	100	0.06	7.08	Clear, colorless solution
	1 day	1.069	97.9	0.471	7.15	Clear, colorless solution
	2 days	1.066	97.6	1.771	7.36	Clear, colorless solution
	6 days	1.043	95.5	4.297	7.12	Clear, colorless solution
	9 days	1.027	94.0	5.648	7.11	Clear, colorless solution
	14 days	1.006	92.1	8.3	7.09	Clear, very slightly yellow sol.
	21 days	0.973	89.1	11.613	7.08	Clear, very slightly yellow sol.
60°C	0time	1.092	100	0.06	7.08	Clear, colorless solution
	1 day	1.028	94.1	6.109	7.12	Clear, colorless solution
	2 days	0.991	90.8	10.291	7.17	Clear, colorless solution
	6 days	0.877	80.3	22.512	7.08	Clear, colorless solution
	9 days	0.806	73.8	28.351	7.06	Clear, yellow solution
	14 days	0.726	66.5	35.59	7.04	Clear, yellow solution
	21 days	0.745	68.2	42.23	6.94	Clear, yellow solution

*Phosphate Buffer: KH_2PO_4 and Na_2HPO_4

Table 10 Stability of MNTX in pH 7, 0.02M Glycine* Solution

Condition	Elapsed Time	Strength (mg/ml)	% Initial	Total Impurities (% Total Area)	pH of Formulation	Appearance and Description
Room Temperature	0time	0.993	100	0.11	7.06	Slightly yellowish, clear solution
	1 day	0.993	100	0.076	6.91	Clear, colorless solution
	2 days	0.994	100.1	0.14	7.11	Clear, colorless solution
	6 days	0.987	99.4	0.302	7.37	Slight precipitate on the bottom
	9 days	1.005	101.2	0.425	7.99	Slightly hazy on the bottom

	14 days	0.998	100.5	0.32	7.21	Slightly hazy on the bottom
	21 days	0.989	99.6	0.62	7.16	Clear, colorless solution
40°C	0time	1.051	100	0.097	7.15	Clear, colorless solution
	1 day	1.04	99.0	0.403	7.53	Clear, colorless solution
	2 days	1.039	98.9	0.379	7.69	Clear, colorless solution
	6 days	1.043	99.2	0.468	7.50	Clear, colorless solution
	9 days	1.039	98.9	0.669	7.16	Clear, colorless solution
	14 days	1.036	98.6	0.74	7.55	Clear, colorless solution
	21 days	1.01	96.1	0.975	7.26	Clear, colorless solution
60°C	0time	1.051	100	0.097	7.15	Clear, colorless solution
	1 day	1.032	98.2	1.046	7.20	Clear, colorless solution
	2 days	1.032	98.2	1.757	7.27	Clear, colorless solution
	6 days	1.002	95.3	4.043	6.98	Clear, colorless solution
	9 days	0.977	93.0	5.294	6.95	Clear, light yellow solution
	14 days	0.959	91.2	6.51	6.94	Clear, light yellow solution
	21 days	0.937	89.2	9.122	6.37	Clear, light yellow solution

*Glycine Buffer: Glycine and NaOH

[00135] Preparation of a methylnaltrexone forumulation comprising sodium EDTA and citrate buffer. Methylnaltrexone formulations consisting of methylnaltrexone, sodium EDTA, and sodium chloride in citrate buffer have been described (see US Patent Application Publication US2004/0266806A1, published December 30, 2004). We have prepared solutions comprising the same components for stability comparison studies with our present formulations.

[00136] Formulations containing 20 mg/mL methylnaltrexone bromide in either A-0.7 mg/mL NaEDTA / pH 3.5 adjusted with citrate buffer; and B- 0.4 mg/mL CaEDTA / 0.65% NaCl / pH 3.5 adjusted with glycine buffer were prepared. Each of the formulations were assessed over time for presence of degradant formation, the results are shown in Table 11.

[00137] Formulations containing 5 mg/mL methylnaltrexone bromide (12 mg/vial or 24 mg/vial) were prepared as described in **Example 12**, below. Each of the formulations were assessed over time for presence of degradant formation, the results are shown in Table 12.

[00138] Under aggressive stability conditions, solutions containing sodium EDTA, even high levels of sodium EDTA, the 0.67 and the 0.79 degradant begin to increase. It is believed the formulations and methods provided herein for production of methylnaltrexone solutions will provide for compositions which retain stability and will maintain acceptable degradant levels over extended time periods.

TABLE 11 Stability Comparisons of 20 mg/mL methylnaltrexone formulation

TABLE 11A. Stability data for liquid formulation containing 20 mg/ml MNTX, 0.7 mg/ml NaEDTA 0.4% Sodium Chloride and pH 3.5 adjusted with Citric buffer (HPLC Method B)

	Initial (mg)	**RRT 0.38**	**RRT 0.49**	**RRT 0.67**	**RRT 0.79**	**RRT 0.89**	**RRT 1.17**	**RRT 1.55**	**RRT 1.66**	**RRT 1.77**	**RRT 1.89**	**RRT 1.96**	**RRT 2.01**	**RRT 2.26**	**Total**
Specifications	NA	0.2	0.5	0.5	0.5	0.15	0.15	0.5	0.15	0.5	0.2	0.2	0.2	0.5	NA
Initial	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
Room Temperature															
Time and Days 7	20.2	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
14	20.0	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
30	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
40°C/75% Relative Humidity															
7	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
14	20.2	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
30	20.0	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
70°C															
7	20.0	BRL	BRL	0.1	0.06	BRL	BRL	BRL	0.13	BRL	BRL	BRL	BRL	0.09	0.38
14	19.9	BRL	BRL	0.16	0.15	BRL	BRL	BRL	0.12	0.06	BRL	BRL	BRL	0.15	0.64
30	20.0	BRL	BRL	0.10	0.38	0.05	BRL	0.10	0.14	BRL	BRL	0.14	BRL	0.30	1.21

Table 11B: *Stability data for liquid formulation 20 mg/ml MNTX, 0.4 mg/ml CaEDTA and 0.65% Sodium Chloride with pH 3.5 adjusted with Glycine Hydrochloride* (HPLC Method B)

	Initial (mg)	RRT 0.38	RRT 0.49	RRT 0.67	RRT 0.79	RRT 0.89	RRT 1.17	RRT 1.55	RRT 1.66	RRT 1.77	RRT 1.89	RRT 1.96	RRT 2.01	RRT 2.26	Total
Specifications	NA	0.2	0.5	0.5	0.5	0.15	0.15	0.5	0.15	0.5	0.2	0.2	0.2	0.5	NA
pH 3.5 at Room Temperature															
Time and Days / Initial	20.2	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
7	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.11
14	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
30	19.8	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
pH 3.5 at 40°C/75% Relative Humidity															
Initial	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
7	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
14	20.0	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
21	20.3	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.11	BRL	BRL	BRL	BRL	BRL	0.11
30	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
pH 3.5 at 70°C															
Initial	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
5	20.2	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.13	BRL	BRL	BRL	BRL	0.08	0.27
7	20.0	BRL	BRL	0.08	0.07	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	0.11	0.38
12	19.9	BRL	BRL	0.06	0.15	BRL	BRL	BRL	0.11	0.06	BRL	BRL	BRL	0.18	0.56

Table 11C-1: Stability Data for Methylnaltrexone Bromide, 20 mg/mL Injection, CaEDTA Formulation

Storage Time		Description Reconstituted Solution	Strength	pH	Edetate Calcium Disodium Content
Specification		Clear solution, colorless to pale yellow, essentially free of visible particulates	90.0 - 110.0 % LC	3.0 - 5.0	0.36 - 0.44 mg/mL
Method		HPLC Method A	L28228-147	USP <791>	L34449-051
Initial		Conforms	98.2, 97.2, 97.6	3.7, 3.6	
25°C/60% RH	1 Month	No change	99.0	3.6, 3.5	0.41
	3 Months	No change	99.1	3.6, 3.6	0.41
	6 Months	No change	100.3	3.4, 3.4	0.41
	9 Months	No change	99.2	3.4, 3.4	0.41
30°C/75%RH	1 Month	No change	99.0	3.5, 3.5	NT
	3 Months	No change	100.1	3.5, 3.5	0.39
	6 Months	No change	100.9	3.4, 3.4	0.40
	9 Months	No change	97.8	3.4, 3.4	0.40
40°C/75% RH Inverted	1 Month	No change	99.1	3.6, 3.6	NT
	3 Months	No change	100.1	3.6, 3.5	0.40
	6 Months	No change	99.9	3.5, 3.5	0.39
40°C/75% RH Upright	1 Month	No change	99.6	3.5, 3.5	NT
	3 Months	No change	100.3	3.5, 3.5	0.40
	6 Months	No change	100.7	3.5, 3.5	0.39
Light Study	Exposed	No change	101.3	3.6	0.40
	Packaged	No change	98.7	3.5	0.40

Table 11C-2: Stability Data for Methylnaltrexone Bromide, 20 mg/mL Injection, CaEDTA Formulation, (Cont'd)

		Degradation/Impurities (HPLC Method A)											
Storage Time		**RRT 0.49**	**RRT 0.89**	**7-Dihydroxy MNTX**	***S*-MNTX**	**Ring Contraction**	**Naltrexone Base**	**2, 2'-*bis* MNTX**	***O*-Methyl MNTX**	**Aldol-Dimer**	**Hofmann Degradation**	**Any Unspecified (Unidentified) Degradant**	**Total Degradants/ Impurities**
Specification		NMT 0.2% w/w	NMT 0.2% w/w	NMT 0.5% w/w	NMT 0.5% w/w	NMT 0.5% w/w	FIO	NMT 0.5% w/w	FIO	NMT 0.5% w/w	NMT 0.5% w/w	NMT 0.2% w/w	NMT 2.0% w/w
Initial		BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
25°C/ 60%RH	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	9 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.08	BRL	BRL	BRL	BRL
30°C/ 75%RH	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	9 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
40°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	0.07	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	0.1
40°C/ 75%RH Upright	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	0.1
	6 months	BRL	BRL	0.07	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	0.1

Table 11C-3 : Stability Data for Methylnaltrexone Bromide, 20 mg/mL Injection, CaEDTA Formulation, (Cont'd)

Storage Time		Degradation/Impurities											
		RRT 0.49	RRT 0.89	7-Dihydroxy MNTX	*S*-MNTX	Ring Contraction	Naltrexone Base [b]	2, 2'-*bis* MNTX	*O*-Methyl[b] MNTX	Aldol-Dimer	Hofmann Degradation	Any Unspecified (Unidentified) Degradant	Total Degradants/ Impurities
Specification		NMT 0.2% w/w	NMT 0.2% w/w	NMT 0.5% w/w	NMT 0.5% w/w	NMT 0.5% w/w	FIO	NMT 0.5% w/w	FIO	NMT 0.5% w/w	NMT 0.5% w/w	NMT 0.2% w/w	NMT 2.0% w/w
Method		HPLC Method A											
Light Study	Exposed	BRL	2.13[c]	BRL	BRL	BRL	BRL	0.56	0.06	BRL	BRL	0.21[c] (RRT1.69), 0.36 (RRT 0.54), 0.22 (RRT 0.62), 0.06(RRT 1.21), 0.09 (RRT 1.41), 0.05 (RRT 1.56), 0.46 (RRT 1.58), 0.07 (RRT2.01), 0.14 (RRT 2.03)	4.4
	Packaged	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL

BRL=Below reporting limit (0.05%); NT=Not tested; NMT=Not more than; RRT=Relative retention time; FIO = For information only.

a. Only one determination for pH was performed (n=1).

b. Process impurities found in the drug substance. Tested for information

c. The unspecified degradant at RRT 1.69 co-elutes with the process impurity *O*-Methylnaltrexone Methobromide. The total degradant reported at RRT 1.69 is 0.27% of which 0.06% is the process impurity *O*-Methylnaltrexone Methobromide and 0.21% is the unspecified degradant/impurity.

TABLE 12 Stability Comparisons of 5 mg/mL (12 mg/vial or 24 mg/vial) methylnaltrexone formulation

Table 12A-1: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(12 mg/vial) IV Solution for Injection, CaEDTA Formulation

Storage Time		Strength	pH	Edetate Calcium Disodium Content
Specification		90.0 - 110.0 % LC	3.0 - 5.0	0.09 0.11g/mL
Method		HPLC Method A	USP <791>	L34449-051
Initial		98.9, 98.3, 98.8	3.6, 3.6	0.094
25°C/60% RH Inverted	1 month	100.1	3.5, 3.5	0.095
	3 months	100.4	3.7, 3.7	0.095
	6 months	99.7	3.6, 3.6	0.097
30°C/75%RH Inverted	1 month	99.9	3.5, 3.5	0.094
	3 months	100.8	3.9, 3.7	0.096
	6 months	99.6	3.6, 3.6	0.099
40°C/75% RH Inverted	1 month	100.2	3.5, 3.6	0.094
	3 months	100.9	3.7, 3.8	0.095
	6 months	100.4	3.7, 3.6	0.097
Light Study	Exposed	103.1	3.7, 3.7	0.091
	Packaged	99.4	3.6, 3.6	0.095

Table 12A-2: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(12 mg/vial) IV Solution for Injection, CaEDTA Formulation, (Cont'd)

		Degradation/Impurities											
Storage Time		RRT 0.49	RRT 0.89	7-Dihydroxy MNTX	*S*-MNTX	Ring Contraction	Naltrexone Base	2, 2'-*bis*	*O*-Methyl MNTX	Aldol-Dimer	Hofmann Degradation	Any Unspecified (Unidentified) Degradant	Total Degradants/ Impurities
Specification		NMT 0.2% w/w	NMT 0.2% w/w	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.4 w/w	FIO	NMT 0.4 w/w	FIO	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.2% w/w	NMT 2.0% w/w
		HPLC Method A											
Initial		BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
25°C/ 60%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
30°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
40°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	0.06
	6 months	BRL	BRL	0.07	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	0.07
Light Study	Exposed	BRL	2.97	0.22	BRL	BRL	BRL	0.28	0.06[c]	BRL	BRL	0.28 (RRT=0.60), 0.08 (RRT=0.63) 0.05 (RRT=0.71), 0.13(RRT=1.21) 0.08 (RRT=1.42), 0.99 (RRT=1.65) 0.31 (RRT=1.71), 0.09 (RRT=2.09)	5.5
	Packaged	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL

BRL=Below reporting limit (0.05%) NT=Not tested NMT=Not more than
RRT=Relative retention time FIO = For information only.

Table 12B-1: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(24mgvial) IV Solution for Injection, CaEDTA Terminally Sterilized

Storage Time		Strength	pH	Edetate Calcium Disodium Content
Specification		90.0 - 110.0 % LC	3.0 - 5.0	0.09 0.11g/mL
Method		HPLC Method A	USP <791>	L34449-051
Initial		99.4, 99.7, 99.7	3.6, 3.7	0.093
25°C/60% RH Inverted	1 month	100.2	3.6, 3.6	0.096
	3 months	100.4	3.6 3.6	0.094
	6 months	99.6	3.7, 3.7	0.096
30°C/75%RH Inverted	1 month	98.7	3.6, 3.6	0.098
	3 months	100.4	3.6, 3.7	0.093
	6 months	100.6	3.7, 3.7	0.096
40°C/75% RH Inverted	1 month	99.5	3.6, 3.6	0.096
	3 months	100.6	3.7, 3.7	0.094
	6 months	100.2	3.7, 3.7	0.094
Light Study	Exposed	100.3	3.7, 3.6	0.095
	Packaged	99.6	3.7, 3.7	0.090

Table 12B-2: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(24mgvial) IV Solution for Injection, CaEDTA Terminally Sterilized (Cont'd)

Storage Time		Degradation/Impurities											
		RRT 0.49	RRT 0.89	7-Dihydroxy MNTX	*S*-MNTX	Ring Contraction	Naltrexone Base	2, 2'-*bis* MNTX	*O*-Methyl MNTX	Aldol-Dimer	Hofmann Degradation	Any Unspecified (Unidentified) Degradant	Total Degradants / Impurities
Specification		NMT 0.2% w/w	NMT 0.2% w/w	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.4 w/w	FIO	NMT 0.4 w/w	FIO	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.2% w/w	NMT 2.0% w/w
		HPLC Method A											
Initial		BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
25°C/ 60%RH Inverted	1 Mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
30°C/ 75%RH Inverted	1 Mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	3 mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
40°C/ 75%RH Inverted	1 Mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 mon	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	0.06
	6 mon	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.08	BRL	BRL	BRL	BRL
Light Study	Exposed	BRL	3.00[a]	0.23[a]	BRL	BRL	BRL	0.29[a]	0.06[c]	BRL	BRL	0.30 (RRT=0.60), 0.08 (RRT=0.63) 0.05 (RRT=0.71), 0.14 (RRT=1.21) 0.09 (RRT=1.42), 0.97 (RRT=1.65) 0.35 (RRT=1.71), 0.09 (RRT=2.09)	5.6
	Packaged	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL

BRL=Below reporting limit (0.05%); NT=Not tested NMT=Not more than; RRT=Relative retention time; FIO = For information only.

Table 12C-1: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(24mgvial) IV Solution for Injection, CaEDTA Formulation

Storage Time		Strength	pH	Edetate Calcium Disodium Content
Specification		90.0 - 110.0 % LC	3.0 - 5.0	0.09 0.11g/mL
Method		HPLC Method A	USP <791>	L34449-051
Initial		99.8, 99.3, 99.2	3.6, 3.6	0.09
25°C/60% RH Inverted	1 month	100.5	3.5, 3.5	0.094
	3 months	100.8	3.7, 3.7	0.095
	6 months	99.8	3.5, 3.5	0.098
30°C/75%RH Inverted	1 month	100.5	3.5, 3.5	0.094
	3 months	100.7	3.7, 3.7	0.095
	6 months	99.9	3.6, 3.6	0.094
40°C/75% RH Inverted	1 month	100.3	3.5, 3.5	0.095
	3 months	100.2	3.8, 3.8	0.095
	6 months	100.3	3.7, 3.6	0.098
Light Study	Exposed	102.6	3.5, 3.6	0.092
	Packaged	99.8	3.6, 3.6	0.095

Table 12C-2: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(24mgvial) IV Solution for Injection, CaEDTA Formulation, (Cont'd)

Storage Time		Degradation/Impurities											
		RRT 0.49	RRT 0.89	7-Dihydroxy MNTX	*S*-MNTX	Ring Contraction	Naltrexone Base	2, 2′-*bis* MNTX	*O*-Methyl MNTX	Aldol-Dimer	Hofmann Degradation	Any Unspecified (Unidentified) Degradant	Total Degradants/ Impurities
Specification		NMT 0.2% w/w	NMT 0.2% w/w	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.4 w/w	FIO	NMT 0.4 w/w	FIO	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.2% w/w	NMT 2.0% w/w
		HPLC Method A											
Initial		BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
25°C/ 60%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
30°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
40°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	0.06
Light Study	Exposed	BRL	2.23[a]	0.19[a]	BRL	BRL	BRL	0.21	0.07	BRL	BRL	0.18 (RRT=0.60), 0.10 (RRT=1.21) 0.06 (RRT=1.42), 1.00 (RRT=1.65) 0.25 (RRT=1.71), 0.07 (RRT=2.09)	4.3
	Packaged	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL

BRL=Below reporting limit (0.05%) NT=Not tested NMT=Not more than
RRT=Relative retention time FIO = For information only.

Table 12D-1: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(24mgvial) IV Solution for Injection, CaEDTA Formulation(Terminally Sterilized) (HPLC Method A)

Storage Time		Strength	pH	Edetate Calcium Disodium Content
Specification		90.0 - 110.0 % LC	3.0 - 5.0	0.09 0.11g/mL
Method		L28228-147	USP <791>	L34449-051
Initial		99.7, 99.8, 98.2	3.5, 3.5	0.095
25°C/60% RH Inverted	1 month	99.7	3.5, 3.5	0.093
	3 months	101.5	3.6, 3.6	0.091
	6 months	100.8	3.6, 3.5	0.095
30°C/75%RH Inverted	1 month	99.9	3.5, 3.5	0.099
	3 months	99.8	3.6, 3.6	0.094
	6 months	101.1	3.6, 3.6	0.094
40°C/75% RH Inverted	1 month	99.5	3.6, 3.6	0.095
	3 months	100.3	3.6, 3.6	0.095
	6 months	100.2	3.7, 3.8	0.095
Light Study	Exposed	103.1	3.7, 3.6	0.093
	Packaged	100.1	3.6, 3.6	0.092

Table 12D-2: Stability Data for Methylnaltrexone Bromide, 5 mg/mL(24mgvial) IV Solution for Injection, CaEDTA Formulation(Terminally Sterilized), (Cont'd)

Storage Time		Degradation/Impurities											
		RRT 0.49	RRT 0.89	7-Dihydroxy MNTX	*S*-MNTX	Ring Contraction	Naltrexone Base	2, 2′-*bis* MNTX	*O*-Methyl MNTX	Aldol-Dimer	Hofmann Degradation	Any Unspecified (Unidentified) Degradant	Total Degradants/ Impurities
Specification		NMT 0.2% w/w	NMT 0.2% w/w	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.4 w/w	FIO	NMT 0.4 w/w	FIO	NMT 0.4 w/w	NMT 0.4 w/w	NMT 0.2% w/w	NMT 2.0% w/w
Method		HPLC Method A											
Initial		BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
25°C/ 60%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
30°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	0.06	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	0.06
40°C/ 75%RH Inverted	1 Month	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL
	3 months	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL
	6 months	BRL	BRL	0.09	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	0.09
Light Study	Exposed	BRL	2.33[a]	0.20[a]	BRL	BRL	BRL	0.24[a]	0.06	BRL	BRL	0.20 (RRT=0.60), 0.05 (RRT=0.63) 0.11 (RRT=1.21), 0.07 (RRT=1.42) 1.08 (RRT=1.65), 0.29 (RRT=1.71) 0.07 (RRT=2.09)	4.6
	Packaged	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.07	BRL	BRL	BRL	BRL

BRL=Below reporting limit (0.05%) NT=Not tested NMT=Not more than
RRT=Relative retention time FIO = For information only.

Example 5

[00139] The stability of a formulation containing 5.0 mg/mL IV (12 mg/vial or 24 mg/vial) was tested to determine the effect of light exposure. The formulations were assessed over time for presence of degradant formation (HPLC Method A). The results of the light stability test is shown in Tables 13A and 13B.

Table 13A: Effect Of Room Light Exposure on The Stability of 5.0 mg/ mL IV (12 mg/Vial): vials filled at ambient condition

Condition	Strength (mg/ml)	RRT 0.63	RRT 0.67 7-dihydroxy MNTX mz 388	RRT 0.79 Contracted Ring	RRT 0.89	RRT 0.91 SMNTX	RRT 1.45 (2,2 BisMNTX)	RRT 1.66 (O-Methyl)	RRT 1.72 Aldol Dimer	Total
5mg/mL (12mg/vial) L34325-122 AS (Aseptically Filled Clear Vials)										
Initial	4.99	BDL	BDL	BDL	BDL	BDL	0.01	0.06	0.04	0.05
5 Days	4.95	BDL	BDL	BDL	BDL	BDL	BDL	0.05	0.03	0.03
10 Days	4.98	BDL	0.04	BDL	BDL	BDL	BDL	0.05	0.04	0.08
16 Days	4.97	BDL	0.03	BDL	BDL	BDL	0.02	0.05	0.03	0.08
5mg/mL (12mg/vial) L34325-122 TS (Terminally Sterilized for 15 minutes Clear vials)										
Initial	5.00	BDL	0.02	BDL	BDL	BDL	0.02	0.06	0.02	0.06
5 Days	4.98	BDL	0.05	BDL	BDL	BDL	0.046	0.05	BDL	0.10
10 Days	4.95	BDL	0.07	BDL	BDL	BDL	0.09	0.05	BDL	0.16
16 Days	4.99	0.01	0.10	BDL	BDL	0.01	0.10	0.06	0.02	0.24
5mg/mL (12mg/vial) L34325-122 AS-AMB (Aseptically Filled Amber Vials)										
Initial	5.21	BDL	0.03	BDL	BDL	BDL	BDL	0.06	0.04	0.07
5 Days	4.95	BDL	BDL	BDL	BDL	BDL	BDL	0.05	0.03	0.03
10 Days	4.96	BDL	BDL	BDL	BDL	BDL	BDL	0.05	0.03	0.03
16 Days	5.01	BDL	BDL	BDL	BDL	BDL	BDL	0.06	0.03	0.03
5mg/mL (12mg/vial) L34325-122 TS_AMB (Terminally Sterilized for 15 minutes Amber vials)										
Initial	5.02	BDL	0.03	0.02	BDL	BDL	0.01	0.06	0.02	0.08
5 Days	4.97	BDL	0.03	BDL	BDL	BDL	BDL	0.06	BDL	0.03
10 Days	5.01	BDL	0.06	BDL	BDL	BDL	BDL	0.05	0.02	0.08
16 Days	4.99	BDL	0.04	0.01	BDL	BDL	0.01	0.06	0.02	0.08

Note: RRT 1.66 (O-Methyl) is not added into the Total
BDL : Below detection limit of 0.01%
BRL: Below Reporting limit of 0.05%

Table 13B: Effect of Room Light Exposure on The Stability of 5.0 mg/ mL IV (24 mg/Vial): vials filled at Ambient condition

Condition	Strength (mg/ml)	RRT 0.63	RRT 0.67 7-dihydroxy MNTX mz 388	RRT 0.79 Contracted Ring	RRT 0.89	RRT 0.91 SMNTX	RRT 1.45 (2,2 BisMNTX)	RRT 1.66 (O-Methyl)	RRT 1.72 Aldol Dimer	Total
5mg/mL (24mg/vial) L34325-122 AS (Aseptically Filled Clear Vials)										
Initial	5.04	BDL	0.01	BDL	ND	BDL	ND	0.05	0.03	0.04
5 Days	5.07	BDL	0.02	BDL	ND	BDL	ND	0.05	0.04	0.06
10 Days	5.00	BDL	0.02	BDL	0.01	BDL	0.02	0.05	0.03	0.08
16 Days	5.03	BDL	0.03	BDL	ND	BDL	0.03	0.06	0.04	0.1
5mg/mL (24mg/vial) L34325-122 TS (Terminally Sterilized for 15 minutes Clear vials)										
Initial	5.01	BDL	0.03	BDL	BDL	BDL	ND	0.06	0.02	0.05
5 Days	5.01	BDL	0.02	BDL	BDL	BDL	0.03	0.06	0.02	0.07
10 Days	5.01	BDL	0.06	BDL	BDL	BDL	0.049	0.06	0.02	0.13
16 Days	5.01	BDL	0.07	BDL	BDL	0.01	0.08	0.06	0.02	0.18
5mg/mL (24mg/vial) L34325-122 AS-AMB (Aseptically Filled Amber Vials)										
Initial	4.99	BDL	0.02	BDL	BDL	BDL	BDL	0.05	0.04	0.06
5 Days	5.01	BDL	BDL	BDL	BDL	BDL	BDL	0.05	0.03	0.03
10 Days	5.01	BDL	0.02	BDL	BDL	BDL	BDL	0.06	0.03	0.05
16 Days	5.02	BDL	BDL	BDL	BDL	BDL	BDL	0.06	0.03	0.03
5mg/mL (24mg/vial) L34325-122 TS_AMB (Terminally Sterilized for 15 minutes Amber vials)										
Initial	4.98	BDL	0.04	BDL	BDL	BDL	BDL	0.06	0.02	0.06
5 Days	5.02	BDL	0.04	BDL	BDL	BDL	BDL	0.06	0.02	0.06
10 Days	5.01	BDL	0.04	BDL	BDL	BDL	BDL	0.05	0.02	0.06
16 Days	5.04	BDL	0.03	BDL	BDL	BDL	BDL	0.05	0.02	0.05

Note: RRT 1.66 (O-Methyl) is not added into the Total
BDL: Below detection limit of 0.01%
BRL: Below Reporting limit of 0.05%

Example 6

Evaluation of Stopper Compatibility

[00140] We assessed various available stoppers used in vial closures for their compatibility with methylnaltrexone solutions, and determined whether any had effects on formation of degradants in solution.

[00141] Identical preparations prepared as described in Example 4 were stored in parallel in vials having either a 13mm WPS S2-F451 4432/50 Gray B2-40 Westar RS stopper (West Pharmaceutical Services) or a 13mm S2-F451 RS D 777-1 RB2 40 stopper (Daikyo Seiko, Ltd)

under various conditions. Each of the stoppers has a FluoroTec® fluorocarbon film; the Westar 4432/50 stopper is chlorobutyl rubber, while the RB2-40 RS D 777-1 stopper is bromobutyl rubber. The presence of accumulation of degradant was assessed for each of the configurations (HPLC Method A). Table 14 depicts the results of these studies. Under accelerated storage conditions, the stopper containing bromobutyl rubber appears to accumulate aldol dimer formation at a higher rate than the comparable chlorobutyl stopper.

TABLE 14: Stopper compatibility evaluation of Methylnaltrexone with 13 mm WPS S2-F451 4432/50 Gray B2-40 Westar RS stopper (West Pharmaceutical Services) and 13 mm S2-F451 RS-D 777-1 B2 40 from Daikyo Seiko Ltd. at Room Temperature and 40°C

	Initial (mg)	RRT 0.38	RRT 0.49	RRT 0.67	RRT 0.79	RRT 0.89	RRT 1.17	RRT 1.55	RRT 1.66	RRT 1.77	RRT 1.89	RRT 1.96	RRT 2.01	RRT 2.26	*Total*
Specifications	NA	0.2	0.5	0.5	0.5	0.15	0.15	0.5	0.15	0.5	0.2	0.2	0.2	0.5	NA
Initial	20.2	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
Control at Room Temperature															
1 hours	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
4 hours	20.0	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
13 mm WPS S2-F451 4432/50 Gray B2-40 Westar RS stopper (West Pharmaceutical Services) at Room Temperature															
1 hours	20.3	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL	BRL	0.06
4 hours	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL	BRL	0.06
13 mm S2-F451 RS-D 777-1 B2 40 from Daikyo Seiko Ltd. at Room Temperature															
1 hours	20.3	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
4 hours	20.2	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
Control at 40°C															
1 hours	20.3	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
4 hours	20.3	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	BRL	BRL	BRL	BRL	BRL	0.12
13 mm WPS S2-F451 4432/50 Gray B2-40 Westar RS stopper (West Pharmaceutical Services) at 40°C															
1 hours	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	BRL	BRL	BRL	BRL	BRL	0.06
4 hours	20.2	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.06	0.05	BRL	BRL	BRL	BRL	0.06
13 mm S2-F451 RS-D 777-1 B2 40 from Daikyo Seiko Ltd. at 40°C															
1 hours	20.1	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	0.05	BRL	BRL	BRL	BRL	0.17
4 hours	19.9	BRL	BRL	BRL	BRL	BRL	BRL	BRL	0.12	0.05	BRL	BRL	BRL	BRL	0.17

Example 7

Stability of Frozen Intravenous Bags

[00142] The following formulation of methylnaltrexone 5 mg/ml, 0.8mg of NaCL, 0.1mg CaEDTA, 0.1 mg Glycine Hydrochloride, and water for injection was infused in 100 ml IV bags

of 0.9% of Normal Saline and frozen at –200C. The study was conducted for two concentrations of methylnaltrexone: 12mg/100 ml and 24 mg/100ml. B/Braun bags NDC 0264-1800-32 with 0.9% of Normal Saline were used.

[00143] Two batches of the formulation were prepared and subjected to stability determination. The first batch was the above methylnaltrexone IV formulation: 5 mg/ml methylnaltrexone, 0.8mg of NaCL, 0.1mg CaEDTA, 0.1 mg Glycine Hydrochloride infused in the 0.9% Normal Saline IV bag. The second batch was just 5 mg/ml methylnaltrexone infused in 0.9% Normal Saline IV bag. The bags were frozen and kept at -20^0C. The stability data showed that over a period of 2 months both batches were stable with no degradants formed. An additional benefit to the frozen bag storage is that no protection from light is required.

[00144] Two months stability study (HPLC Method A) showed no degradation was formed thereby demonstrating that the formulation is stable under frozen conditions, that the period of use and shelf life can be longer than 6 months, and that there is no need for the hospital staff to infuse the IV bags with the drug. The bags come user ready only need to be thawed. Table 15 summarizes the results of these studies.

Table 15: 5 mg/ml Methylnaltrexone, 0.8mg of NaCL, 0.1mg CaEDTA, 0.1 mg Glycine HCl

12 mg/100 ml of 0.9% Normal Saline bag

Sample name	Strength, mg/ml	Impurities						
		RRT 0.67	RRT 0.79	RRT 0.89	RRT 1.55	RRT 1.76	RRT 2.24	Total
Initial	0.11	ND	ND	ND	ND	ND	ND	NA
2 weeks	0.11	ND	ND	ND	ND	ND	ND	NA
1 month	0.11	ND	ND	ND	ND	ND	ND	NA
2 months	0.12	ND	ND	ND	ND	ND	ND	NA

24 mg/100 ml bag of 0.9% Normal Saline bag

Sample name	Strength, mg/ml	Impurities						
		RRT 0.67	RRT 0.79	RRT 0.89	RRT 1.55	RRT 1.76	RRT 2.24	Total
Initial	0.22	ND	ND	ND	ND	0.07	ND	0.07
2 weeks	0.22	ND	ND	ND	ND	0.07	ND	0.07
1 month	0.23	ND	ND	ND	ND	0.06	ND	0.06
2 months	0.23	ND	ND	ND	ND	0.06	ND	0.06

Example 8

[00145] The effect of sodium tungstate (HPLC Method A) on the subcutaneous formulation described herein is summarized in Table 16, below.

Table 16. Effect of 1mM Sodium Tungstate on Subcutaneous Formulation

Sample	7-Dihydroxy MNTX	Ring Contraction Degradant	O-Methyl MNTX	Total
Room Temperature				
Methylnaltrexone Initial	BRL	BRL	0.12	0.12
Methylnaltrexone 1 hour	BRL	BRL	0.12	0.12
Methylnaltrexone 2 hours	0.02	BRL	0.12	0.14
Methylnaltrexone 3 hours	0.02	BRL	0.12	0.14
Methylnaltrexone 4 hours	0.02	BRL	0.12	0.14
Methylnaltrexone 5 hours	0.02	BRL	0.12	0.14
Methylnaltrexone +1mM Sodium Tungstate Initial	BRL	BRL	0.12	0.12
Methylnaltrexone +1mM Sodium Tungstate 1 hour	0.02	BRL	0.12	0.14
Methylnaltrexone +1mM Sodium Tungstate 2 hours	0.02	BRL	0.12	0.14
Methylnaltrexone +1mM Sodium Tungstate 3 hours	0.03	BRL	0.12	0.15
Methylnaltrexone +1mM Sodium Tungstate 4 hours	0.03	BRL	0.12	0.15
Methylnaltrexone +1mM Sodium Tungstate 5 hours	0.03	BRL	0.12	0.15
40^0C				
Methylnaltrexone Initial	BRL	BRL	0.12	0.12
Methylnaltrexone 1 hour	BRL	BRL	0.12	0.12
Methylnaltrexone 2 hours	0.02	BRL	0.12	0.14
Methylnaltrexone 3 hours	0.02	BRL	0.12	0.14
Methylnaltrexone 4 hours	0.02	BRL	0.12	0.14
Methylnaltrexone 5 hours	0.03	BRL	0.12	0.15
Methylnaltrexone +1mM Sodium Tungstate Initial	BRL	BRL	0.12	0.12
Methylnaltrexone +1mM Sodium Tungstate 1 hour	0.02	BRL	0.12	0.14
Methylnaltrexone +1mM Sodium Tungstate 2 hours	0.02	BRL	0.12	0.14
Methylnaltrexone +1mM Sodium Tungstate 3 hours	0.03	BRL	0.12	0.15
Methylnaltrexone +1mM Sodium Tungstate4 hours	0.03	BRL	0.12	0.15
Methylnaltrexone +1mM Sodium Tungstate 5 hours	0.03	BRL	0.12	0.15

PART II: Subcutaneous Formulations

Example 9

[00146] A room temperature methylnaltrexone formulation 20 mg/mL subcutaneous solution for injection, CaEDTA formulation consists of 20 mg/mL methylnaltrexone bromide, 0.4 mg/mL edetate calcium disodium (CaEDTA), 0.3 mg/mL glycine hydrochloride and 0.65% sodium chloride in water for injection. The product, which is stable at room temperature storage conditions, is filled aseptically in single-use vials at 0.6 mL volume or 12 mg methylnaltrexone per vial to be administered subcutaneously.

[00147] The sodium chloride concentration is adjusted to 0.65% to maintain the tonicity of the formulation.

[00148] Such a room temperature formulation for subcutaneous administration was prepared as summarized in Tables 17A, 17B, and 17C below:

Table 17A: Methylnaltrexone 20 mg/mL Subcutaneous Solution for Injection,

		SC Commercial
Formulation	**Strength**	**20 mg/mL**
	Type	**Liquid Solution**
Container/Closure	**Vial**	3 mL
	Stopper	13 mm
mg / vial	**Methylnaltrexone**	12 mg
	CaEDTA	0.32
	Glycine HCl	0.24
	NaCl	5.20
	Overage	33% (0.2ml)
Processing	**Sterilization**	Aseptic
	Nitrogen Flush	Yes
	Fill Volume	0.8 mL
Dispensing	**Container**	Syringe
	Dilution	None

Table 17B: Methylnaltrexone 20 mg/mL Subcutaneous Solution for Injection,

	Room Temperature
MNTX	20 mg/mL
CaEDTA[#]	0.40 mg/mL
Glycine HCL	0.30 mg/mL
NaCl	6.5 mg/mL
Osmolarity (mOsm/Kg)	286
pH	3 -5
Volume of injection (mL)	0.6

Table 17C: Methylnaltrexone 20 mg/mL Subcutaneous Solution for Injection,

Quantitative Composition
Methylnaltrexone 20 mg/mL Subcutaneous Solution for Injection, CaEDTA Formulation, Batch Size: 5000 mL

Ingredient	**% WT/WT**	**Input/ Dosage Unit**	
		Input	**Unit**
Naltrexone Methobromide	1.985	16	mg
Calcium EDTA, USP	0.040	0.32	mg
Sodium Chloride, USP	0.644	5.2	mg
Glycine Hydrochloride	0.030	0.24	mg
Water for Injection, USP	NA	QS to 0.80	mL
Hydrochloric Acid, NF[b]	N/A	N/A	
Sodium Hydroxide, NF[b]	N/A	N/A	

[00149] In certain embodiments, the above formulation for subcutaneous administration may be dosed according to the following table. Patients whose weight falls outside the recited ranges may be dosed at 0.15 mg/kg.

Patient Weight		**Injection Volume**	**Dose**
Pounds	Kilograms		
84 to less than 136	38 to less than 62	**0.4 mL**	8 mg
136 to 251	62 to 114	**0.6 mL**	12 mg

[00150] In other embodiments, in patients with severe renal impairment (creatinine clearance less than 30 mL/min) the above formulation for subcutaneous administration dose may be reduced by one-half.

Example 10

[00151] As described herein, the present invention provides a pre-filled syringe containing a methylnaltrexone formulation in accordance with the present invention. Such a pre-filled syringe is described below in Table 18.

Table 18: Pre-filled Syringe

Active Ingredients	**Concentration / Limits**	
Methylnaltrexone Bromide	20 mg/mL	
Excipients	***Concentration / Limits***	
Calcium Disodium Edetate	0.4 mg/mL	
Glycine Hydrochloride	0.3 mg/mL	
Sodium Chloride	6.5 mg/mL	
Water for Injection (WFI)	Ad 1.0 mL	
Primary Packaging Materials	**Type**	**Material**
SCF Syringe 1 mL-1 with Rigid Needle Shield (RNS)	BD	**Glass:** Type 1 **Needle:** Stainless steel AISI 304, CN18/10, 27G1/2, 5-bevel **Soft needle shield:** FM27/0 modified **Rigid shell:** Polypropylene
SCF Stopper	BD	**Basic raw material:** bromobutyl rubber, 4023/50, grey **Coating:** contact side with Daikyo foil, remaining part: B2-40 coated

Example 11

Subcutaneous Formulation - Bioequivalency Study

[00152] A bioequivalency study comparing the subcutaneous formulation described at Example 9 and a formulation containing only methylnaltrexone in saline was performed in an open-label, single-dose, randomized, 2-period, 2-sequence crossover,

inpatient/outpatient study in healthy subjects conducted at a single investigational site. Doses were administered after an overnight fast of at least 10 hours. Healthy men and nonlactating and nonpregnant women aged 18 to 50 years were eligible for enrollment if all other qualifying criteria were met. At approximately 0800 on day 1 of periods 1 and 2, each subject received an SC injection containing 0.15 mg/kg of methylnaltrexone (the period 1, day -1 weight was used to determine the dose to be administered). Standard medium fat-meals, served according to the clinic's schedule, could start 3 hours after test article administration. Vital signs, ECGs, laboratory evaluations, and pharmacokinetic (PK) sample collection were completed at designated times on days 1, 2, and 3 of period 1 and 2 as per the study flowchart.

[00153] Each subject was to receive a single SC dose of 0.15 mg/kg of the assigned formulation of methylnaltrexone on day 1 of each period after an overnight fast of at least 10 hours. The injection was administered SC into the upper arm and the same arm was to be used for each injection. The injection site was to be healthy appearing skin. Every attempt was made to have the same person administer both formulations to each subject. The dose was determined from the subject's weight on day -1 of period 1. The syringes were weighed before and after test article administration to verify the volume injected. Each single dose was separated by a washout interval of at least 7 days. Blood samples were obtained for the determination of the pharmacokinetics of methylnaltrexone. Blood samples (6 mL) were collected from an indwelling catheter or by direct venipuncture. If a catheter was used for blood collection, then approximately 0.5 mL of blood were to be discarded before collecting the sample at each sampling time. Blood samples were collected in each period on day 1 within 2 hours before test article administration and at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours after test article administration. Results of pharmaceokinetic studies are set forth in Table 19, below.

Table 19 Methylnaltrexone Pharmacokinetic Parameters for SC Methylnaltrexone Formulations in 27 Healthy Subjects at a Dose of 0.15 mg/kg

Formulation	C_{max} ng/mL	AUC_t ng h/ml	AUC_∞ ng h/ml	$T_{1/2}$ (h)	t_{max} (h)
Saline (min, max)	119 ± 33 (62.6, 197)	221 ± 36 (163, 333)	223 ± 36 (168, 335)	9.2 ± 2.5 (7.0, 19.4)	0.41 (0.08, 1.0)
Example 9 (min, max)	127 ± 34 (82.9, 188)	218 ± 37 (165, 333)	220 ± 37 (172, 335)	8.4 ± 1.4 (6.4, 13.8)	0.34 (0.08, 1.0)

[00154] As shown in Table 19 above, the mean methylnaltrexone concentration-versus-time profile after the SC administration of a formulation of Example 9 was essentially identical to that seen with a saline formulation. Plasma methylnaltrexone concentrations increased sharply in response to SC administration of either formulation, with a mean Cmax of 127 ng/mL for a provided formulation and 119 ng/mL for the saline formulation, observed mostly within the first hour (mean tmax of 0.34 h and 0.41 h, respectively).

Example 12

Pharmacokinetic Screening of Methylnaltrexone Subcutaneous Formulation in Dogs

[00155] Three different methylnaltrexone formulations administered subcutaneously were evaluated in dogs. Pharmacokinetics of methylnaltrexone following a single subcutaneous 0.15 mg/kg dose in male beagle dogs. Eight male dogs (9.4-15 kg) were divided into two groups, four dogs per group. To both groups of dogs, 0.15 mg/kg methylnaltrexone in normal saline (Batch 1) was administered subcutaneously as a reference formulation during period 1. A week later, during period 2, Group 1 (SAN 1-4) received 0.15 mg/kg methylnaltrexone subcutaneously in saline containing 0.5 mg/vial Na. EDTA and 0.6 mM Citrate (Batch 2) and Group 2 (SAN 5-8) received 0.15 mg/kg methylnaltrexone subcutaneously in saline containing 0.5 mg/vial Ca. EDTA (Batch 3). Blood samples were drawn at 0 (predose), 0.0833, 0.167, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 12 hours after dosing, plasma was separated and assayed for methylnaltrexone content.

[00156] Bioanalytical results were obtained, and pharmacokinetic (PK) assessment was performed. Individual dog plasma methylnaltrexone concentration-time profiles were subjected to noncompartmental PK analyses (WinNonlin, Model 200). The

following pharmacokinetic parameters were determined for each dog, and descriptive statistics were calculated for comparison among formulations: AUC, C_{max}, t_{max} and $t_{1/2}$. See Table 20.

Table 20: Individual and Mean (± SD) Dog Plasma Methylnaltrexone Pharmacokinetic Parameters After a Single Subcutaneous Administration (~ 0.15 mg/kg) of Three Injectable Formulations

Formulation	Batch	1	2		Batch	1	3	
				Test/				Test/ Reference
	SAN	Reference	Test	Reference	SAN	Reference	Test	
Dose	Mean	0.149	0.150	1.01	Mean	0.152	0.154	1.02
AUC_{0-12}	Mean	87.8	98.9	1.12	Mean	85.4#	90.5	0.97
(hr·ng/mL)	SD	10.7	30.8	0.24	SD	5.1#	20.5	0.15
$AUC_{0-\infty}$	Mean	102	111	1.09	Mean	106	112	0.99
(hr·ng/mL)	SD	9.4	27.9	0.19	SD	9.3	21.2	0.12
AUC_{0-12}	Mean	590	656	1.11	Mean	570#	585	0.95
/Dose	SD	64.2	178	0.22	SD	45.0#	122	0.15
C_{max} (ng/mL)	Mean	83.7	107	1.35	Mean	128#	130	1.01
	SD	33.8	44.4	0.50	SD	22.5#	34.6	0.42
T_{max} (hr)	Mean	0.33	0.19	0.71	Mean	0.19#	0.15	0.92
	SD	0.19	0.04	0.34	SD	0.05#	0.08	0.44
$t_{1/2}$ (1/hr)	Mean	10.1*	9.3*	1.15	Mean	13.0*	13.8*	1.20
	SD	5.0	3.3	0.79	SD	4.0	3.5	0.19

PART III: Intravenous Formulations

Example 12

[00157] In certain embodiments, the present invention provides a methylnaltrexone formulation for intravenous administration. Provided intravenous formulations can be prepared in 12 mg/vial or 24 mg/vial concentrations. Both 12 mg/vial and 24 mg/vial strengths use a 5 mg/mL concentration of methylnaltrexone. In certain embodiments, provided intravenous formulations utilize a 10 mL spikable vial designed to be used with Baxter mini-bags or any other spikable infusion system. In some embodiments, provided formulations were subjected to terminal sterilization by heating at 121°C for 15 minutes.

[00158] Formulations prepared in 12 mg/vial or 24 mg/vial concentrations are set forth in Tables 20A and 20B, respectively, below. Such formulations can be administered at doses of 24 mg, or also, for example, 0.3 mg/kg, every 6 hours as a 20-minute infusion. In certain embodiments, such administration is continued for 3 days (total of 12 doses). Each methylnaltrexone formulation is diluted to 50 mL and administered using a calibrated pump.

Table 20A: Methylnaltrexone IV formulation for 12 mg/Vial

Ingredient	% WT/WT	Input/ Dosage Unit Input	Unit
Naltrexone Methobromide	0.496	25.2	mg
Calcium EDTA, USP	0.0099	0.504	mg
Sodium Chloride, USP	0.833	42.336	mg
Glycine Hydrochloride	0.0099	0.504	mg
Water for Injection, USP	NA	QS to 2.54	mL

		IV	
Formulation	**Strength**	**5 mg/mL**	
	Type	**Liquid Solution**	
Container/Closure	**Vial**	10 mL	10 mL
	Stopper	20 mm	20 mm
mg / vial	**Methylnaltrexone**	12 mg	24 mg
	CaEDTA	0.24 mg	0.48 mg
	Glycine HCl	0.24 mg	0.48 mg
	NaCl	20.16 mg	40.32 mg
	Overage	5%	5%
Processing	**Sterilization**	Terminal	Terminal

	Nitrogen Flush	No *	No *
	Fill Volume	2.52ml (12mg/vial);	5.04ml (24mg/vial)
Dispensing	**Container**	Syringe / spike	Syringe / spike
	Dilution	Dilution / admix	Dilution / admix

Table 20B: Methylnaltrexone IV formulation for 24 mg/Vial

DESCRIPTION	AMT. NEEDED PER UNIT	
Methylnaltrexone	25.2	mg
Calcium EDTA, USP	0.504	mg
Sodium Chloride, USP	42.336	mg
Glycine Hydrochloride	0.504	mg
Water for Injection, USP[a]	5.08[c]	g
Hydrochloric Acid, NF[b]	As needed	NA
Sodium Hydroxide, NF[b]	As needed	NA
Containers & Closures		
10 mL Schott flint glass vial with 20 mm neck		
20 MM, GREY, S10-F451 4432/50		
FLUROTEC PLUG XKD484		
20 mm, Aluminum seal with Flip-top		

Ingredient	% WT/WT	Input/ Dosage Unit Input	Unit
Methylnaltrexone	0.496	25.2	mg
Calcium EDTA, USP	0.0099	0.504	mg
Sodium Chloride, USP	0.833	42.336	mg
Glycine Hydrochloride	0.0099	0.504	mg
Water for Injection, USP	NA	QS to 5.04	mL

[00159] In certain embodiments, fill volume is at least 2.6 mL for a 2.4 mL extractable volume, and at least 5.1 mL for a 4.8 mL extractable volume. Table 20C below describes vial contents dilution when using a traditional syringe or a spikable vial.

Table 20C: Overage and Reconstitution of Sample

	spikable technique with Baxter Mini-bag		traditional syringe withdrawal	
Concentration	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL
mg/vial	12 mg	24 mg	12 mg	24 mg
Overage	5%	5%	5%	5%
Fill volume	2.52	5.04	2.52	5.04
Reconstitution volume	**8.0** mL of saline solution	**5.0** mL of saline solution	**8.0** mL of saline solution	**5.0** mL of saline solution
Withdrawal amount	Spike full contents of vial	Spike full contents of vial	Withdraw 10.0 mL via syringe	Withdraw 10.0 mL via syringe

Example 14

[00160] In certain embodiments, a provided intravenous formulation is administered to a patient 90 minutes post surgery, where the surgery is hernia repair. In some embodiments, the hernia repair patient is administered opioids *via* PCA pump. Such formulations can be administered at doses of 12 mg or 24 mg, or also, for example, 0.3 mg/kg, every 6 hours as a 20-minute infusion. In certain embodiments, such administration is continued for 10 days, the patient is discharged, or 24 hours post- bowel movement.

[00161] One skilled in the art will readily ascertain the essential characteristics of the invention, and understand that the foregoing description and examples are illustrative of practicing the provided invention. Those skilled in the art will be able to ascertain using no more than routine experimentation, many variations of the detail presented herein may be made to the specific embodiments of the invention described herein without departing from the spirit and scope of the present invention.

[00162] Patents, patent applications, publications, and the like are cited throughout the application. The disclosures of each of these documents are incorporated herein by reference in their entirety.

MCSLLP

CLAIMS

1. A pharmaceutical composition consisting of an effective amount of methylnaltrexone, or a pharmaceutically acceptable salt thereof, a calcium salt chelating agent, an isotonic agent, and a glycine stabilizing agent, in an aqueous solution wherein the solution has a pH of between about 2.5 and about pH 6.

2. The composition according to claim 1, wherein the methylnaltrexone is methylnaltrexone bromide.

3. The composition according to claim 2, wherein the methylnaltrexone bromide is provided at a concentration of 20 mg/mL.

4. The composition according to any one of claims 1 to 3, wherein the calcium salt chelating agent is calcium EDTA disodium.

5. The composition according to any one of claims 1 to 4, wherein the isotonic agent is sodium chloride.

6. The composition according to any one of claims 1 to 5, wherein the glycine stabilizing agent is glycine hydrochloride.

7. The composition according to any one of claims 1 to 6, wherein the solution has a pH of about 3.5.

8. A pharmaceutical composition comprising methylnaltrexone bromide, calcium EDTA disodium, sodium chloride, and glycine hydrochloride, in an aqueous solution wherein the solution has a pH of between 2.5 and pH 6.

9. The composition according to any one of claims 2 to 8, wherein the methylnaltrexone bromide is provided at a concentration of 20 mg/ L.

10. The composition according to any one of claims 4 to 9, wherein the calcium EDTA disodium is provided at a concentration of about 0.1 to about 1.0 mg/mL.

11. The composition according to claim 10, wherein the calcium EDTA disodium is provided at a concentration of about 0.4 mg/mL.

12. The composition according to any one of claims 6 to 9, wherein the glycine hydrochloride is provided at a concentration of about 0.05 to about 1.0 mg/mL.

13. The composition according to claim 12, wherein the glycine hydrochloride is provided at a concentration of about 0.3 mg/mL.

14. The composition according to claim 8, wherein the methylnaltrexone bromide is provided at a concentration of about 20 mg/mL, the calcium EDTA disodium is provided at a concentration of about 0.4 mg/mL, the glycine hydrochloride is provided at a concentration of about 0.3 mg/mL, and the solution has a pH of about 2.6 to about 5.0.