# PHARMACEUTICAL COMPOSITION OF S-KETAMINE HYDROCHLORIDE

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U. S. Provisional Application 61/791,237, filed on March 15, 2013, which is incorporated by reference herein in its entirety.

## FIELD OF THE INVENTION

The present invention is directed to an aqueous formulation of S-ketamine hydrochloride, preferably for nasal administration, wherein the formulation does not contain an antimicrobial preservative.

## BACKGROUND OF THE INVENTION

Ketamine (a racemic mixture of the corresponding S- and R- enantiomers) is an NMDA receptor antagonist, with a wide range of effects in humans, including analgesia, anesthesia, hallucinations, dissociative effects, elevated blood pressure and bronchodilation. Ketamine is primarily used for the induction and maintenance of general anesthesia. Other uses include sedation in intensive care, analgesia (particularly in emergency medicine) and treatment of bronchospasms. Ketamine has also been shown to be efficacious in the treatment of depression (particularly in those who have not responded to current anti-depressant treatment). In patients with major depressive disorders, ketamine has additionally been shown to produce a rapid antidepressant effect, acting within hours.

The *S*-ketamine enantiomer (or *S*-(+)-ketamine or esketamine) has higher potency or affinity for the NMDA receptor and thus potentially allowing for lower effective dosages; and is available for medical use, administered either IV (intravenously) or IM (intramuscularly), under the brand name KETANEST S.

In the formulation of a pharmaceutical composition, the stability of the active ingredient is a primary concern. In general, drug substances are less stable in aqueous media than solid dosage forms, and it is important to properly stabilize

and preserve liquid aqueous formulations such as solutions, suspensions, and emulsions. Acid-base reactions, acid or base catalysis, oxidation, and reduction can occur in these products. These reactions can arise from drug substance-excipient interactions, excipient-excipient interactions or container-product interactions. Particularly for pH sensitive compounds, these interactions may alter the pH and may decrease solubility and potentially cause precipitation.

Oxidative labile drug substances or vitamins, essential oils, and almost all fats and oils can be oxidized by auto-oxidation. Such reactions can be initiated by heat, light, peroxides, or other labile compounds or heavy metals such as copper or iron.

The effect of trace metals can be minimized by using chelating agents such as EDTA. Antioxidants may retard or delay oxidation by rapidly reacting with free radicals as they are formed (quenching). Common antioxidants include propyl, octyl and dodecylesters of gallic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid, sodium ascorbate, monothioglycerol, potassium or sodium metabisulfite, propionic acid, propyl gallate, sodium bisulfite, sodium sulfite, and the tocopherols or vitamin E.

In addition to stabilization of pharmaceutical preparations against chemical and physical degradation, liquid and semisolid preparations, particularly multiple dosed preparations, must usually be protected against microbial contamination. In contrast to solid preparations, aqueous solutions, syrups, emulsions, and suspensions often provide excellent growth media for microorganisms such as molds, yeast, and bacteria (e.g. *Pseudomonas Aeruginosa*, *E. Coli*, *Salmonella spp.*, *Staphylococcus aureus, Candida albicans, Aspergillus niger*). Contamination by these microorganisms may occur during manufacturing or when a dose is taken from a multiple dosed formulation. Growth of the microorganisms occurs when a sufficient amount of water is present in the formulation.

Ophthalmic and injectable preparations are typically sterilized by autoclaving or filtration. However, many of them require the presence of an antimicrobial preservative to maintain aseptic conditions throughout their stated shelf life, specifically for multiple dosed preparations.

When a preservative is required, its selection is based upon several considerations, in particular the site of use whether internal, external or ophthalmic (for further details it can be referred to e.g. Remington, The Science and Practice of Pharmacy, 21st edition, Lippincott Williams & Wilkins, 2005).

Many liquid formulations for oral administration, particularly multiple dosed formulations, contain parabens as preservatives, e.g. methyl paraben (methyl-4-hydroxybenzoate) and propyl paraben (propyl-4-hydroxybenzoate). For example, in the Federal Republic of Germany liquid oral formulations containing parabens are commercialized under the trademarks: ben-u-ron®; Cetirizin-ratiopharm®; Pipamperon HEXAL®; Sedotussin®; TALOXA®; Truxal®; XUSAL®; talvosilen®; and Timonil®. Other commercialized liquid formulations contain sorbic acid or its potassium salt as preservative, e.g. ibuprofen liquid formulations and morphine liquid formulations.

Because of the number of excipients and additives in these preparations, it is recommended all the ingredients be listed on the container to reduce the risks that confront hypersensitive patients when these products are administered.

The preservatives benzalkonium chloride and potassium sorbate are also widely used e.g. in nasal drops and sprays. Recently, side effects resulting from mucosal damage caused by benzalkonium chloride and potassium sorbate were reported (cf. C.Y. Ho et al., Am J Rhinol. 2008, 22(2), 125-9). As far as hypersensitivity reactions of preservatives in topical ophthalmic therapies are concerned, quaternary ammoniums (benzalkonium chloride) are commonly associated with irritant toxic reactions whereas the organomercurials (thimerosal) and the alcohols (chlorobutanol) have high associations, respectively, with allergic responses (cf. J. Hong et al., Curr Opin Allergy Clin Immunol. 2009, 9(5), 447-53). Parabens have been implicated in numerous cases of contact sensitivity associated with cutaneous exposure (cf. M.G. Soni et al., Food Chem Toxicol. 2001, 39(6), 513-32) and have been reported to exert a weak estrogenic activity (cf. S. Oishi, Food Chem Toxicol. 2002, 40(12), 1807-13 and M.G. Soni et al., Food Chem Toxicol. 2005, 43(7), 985-015).

Due to these undesired side effects of known preservatives, it is desirable to provide aqueous pharmaceutical compositions (for example, for nasal administration) that exhibit a sufficient shelf life and in use stability in the absence of preservatives or at least in the presence of comparatively low quantities thereof.

## SUMMARY OF THE INVENTION

The present invention is directed to a pharmaceutical composition of S-ketamine hydrochloride, comprising S-ketamine hydrochloride and water; wherein the pharmaceutical composition does not contain an antimicrobial preservative.

In an embodiment, the present invention is directed to a pharmaceutical composition of S-ketamine hydrochloride; wherein the formulation does not contain an antimicrobial preservative; and wherein the pharmaceutical composition is formulated for nasal administration.

The present invention is further directed to a pharmaceutical composition of S-ketamine hydrochloride; wherein the formulation does not contain an antimicrobial preservative; and wherein the pharmaceutical composition further comprises a penetration enhancer, preferably TUDCA.

In another embodiment, the present invention is directed to a pharmaceutical composition of S-ketamine hydrochloride; wherein the formulation does not contain an antimicrobial preservative; and wherein the pharmaceutical composition further comprises TUDCA; wherein the TUDCA is present in a concentration in the range of from about 1.0 mg/mL to about 25 mg/mL.

## DETAILED DESCRIPTION OF THE INVENTION

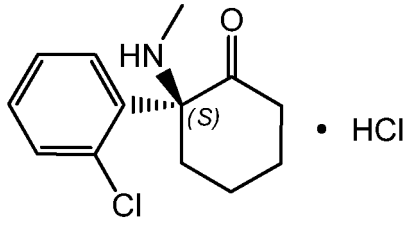
The present invention is directed to pharmaceutical composition of S-ketamine, wherein the pharmaceutical composition does not contain an antimicrobial preservative.

The pharmaceutical compositions of the present invention are based on the unexpected finding that S-ketamine hydrochloride exhibits preservative properties. Thus, when formulating pharmaceutical compositions of S-ketamine hydrochloride, particularly aqueous liquid compositions, preservatives can be completely omitted

while still achieving desired shelf life. Further, although the pharmaceutical compositions of S-ketamine hydrochloride of the present invention do not contain an antibacterial preservative, said compositions do not need to manufactured under aseptic conditions and / or do not need to be sterilized after production.

Additionally, wherein the pharmaceutical compositions of the present invention are formulated for nasal administration, the absence of preservatives results in the elimination of adverse effects associated with said preservatives, including for example, irritation or damage of the mucosal membrane.

As used herein, unless otherwise noted, the terms "**S-ketamine**", "**S-ketamine hydrochloride**" and "**esketamine**" shall mean the (S)-enantiomer of ketamine, as its corresponding hydrochloride salt, a compound of formula (I)




(I)

also known as (*S*)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride.

As used herein, the term "**composition**" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts.

The term "**pharmaceutical composition**" includes any pharmaceutical preparation or formulation that is customized for being administered to a human being or animal. Preferably, the composition contains one or more physiologically acceptable carriers and/or excipients. Preferably, the pharmaceutical compositions of the present invention contain water.

To prepare a pharmaceutical composition of the present invention, S-ketamine hydrochloride as the active ingredient is intimately admixed with a pharmaceutical carrier, preferably water, according to conventional pharmaceutical compounding techniques, which carrier may take a wide variety of forms depending of the form of preparation desired for administration. Suitable pharmaceutically acceptable carriers are well known in the art. Descriptions of some of these pharmaceutically acceptable carriers may be found in The Handbook of Pharmaceutical Excipients, published by the American Pharmaceutical Association and the Pharmaceutical Society of Great Britain.

Methods of formulating pharmaceutical compositions have been described in numerous publications such as Pharmaceutical Dosage Forms: Tablets, Second Edition, Revised and Expanded, Volumes 1-3, edited by Lieberman et al; Pharmaceutical Dosage Forms: Parenteral Medications, Volumes 1-2, edited by Avis et al; and Pharmaceutical Dosage Forms: Disperse Systems, Volumes 1-2, edited by Lieberman et al; published by Marcel Dekker, Inc.

In an embodiment, the present invention is directed to an aqueous formulation of S-ketamine, comprising water and S-ketamine; wherein the S-ketamine is present in an amount in the range of from about 100 mg/mL to about 250 mg/mL, or any amount or range therein, based on the total volume of the pharmaceutical composition. Preferably, the S-ketamine is present in an amount in the range of from about 150 mg/ml to about 200 mg/mL, or any amount or range therein. More preferably, the S-ketamine is present in an amount in the range of from about 150 mg/mL to about 175 mg/mL, or any amount or range therein. More preferably, the S-Ketamine is present in an amount in the range of from about 160 mg/mL to about 163 mg/mL, for example, in an amount of about 161.4 mg/mL.

In an embodiment, the present invention is directed to an aqueous formulation of S-ketamine, comprising water and S-ketamine; wherein the S-ketamine is present in an amount in the range of from about eq. 100 mg/mL to about eq. 250 mg/mL, or any amount or range therein, based on the total volume

of the pharmaceutical composition. Preferably, the S-ketamine is present in an amount in the range of from about eq. 125 mg/ml to about eq. 180 mg/mL, or any amount or range therein. More preferably, the S-ketamine is present in an amount in the range of from about eq. 140 mg/mL to about eq. 160 mg/mL, or any amount or range therein, for example, in an amount of about eq. 140 mg/mL.

The pharmaceutical compositions according to the invention are preferably an aqueous formulation. As used herein, unless otherwise noted, the term "**aqueous**" shall mean that the primary liquid component of the formulation is water. Preferably, water constitutes greater than about 80 wt% of the liquid component of the pharmaceutical composition, more preferably greater than about 90 wt%, more preferably greater than about 95 wt%, more preferably about 98 wt%.

In an embodiment of the present invention, the water content of the composition is within the range of 85±14 wt.-%, more preferably 85±12 wt.-%, still more preferably 85±10 wt.-%, most preferably 85±7.5 wt.-% and in particular 85±5 wt.-%, based on the total weight of the composition.

In another embodiment of the present invention, the water content of the composition is within the range of 90±14 wt.-%, more preferably 90±12 wt.-%, still more preferably 90±10 wt.-%, most preferably 80±7.5 wt.-% and in particular 90±5 wt.-%, based on the total weight of the composition.

In another embodiment of the present invention, the water content of the composition is within the range of 95±4.75 wt.-%, more preferably 95±4.5 wt.-%, still more preferably 95±4 wt.-%, yet more preferably 95±3.5 wt.-%, most preferably 95±3 wt.-% and in particular 95±2.5 wt.-%, based on the total weight of the composition.

In another embodiment of the present invention, the water content of the composition is within the range of from 75 to 99.99 wt.-%, more preferably 80 to 99.98 wt.-%, still more preferably 85 to 99.95 wt.-%, yet more preferably 90 to 99.9 wt.-%, most preferably 95 to 99.7 wt.-% and in particular 96.5 to 99.5 wt.-%, based on the total weight of the composition.

In another embodiment, the pharmaceutical compositions of the present invention further comprises one or more buffers and / or buffer systems (i.e. conjugate acid-base-pairs).

As used herein, the term "**buffer**" shall mean any solid or liquid composition (preferably an aqueous, liquid composition) which when added to an aqueous formulation adjusts the pH of said formulation. One skilled in the art will recognize that a buffer may adjust the pH of the aqueous formulation in any direction (toward more acidic, more basic or more neutral pH). Preferably, the buffer is pharmaceutically acceptable.

Suitably examples of buffers which may be used in the aqueous formulations of the present invention include, but are not limited to citric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, acetic acid, boric acid, sodium borate, succinic acid, tartaric acid, malic acid, lactic acid, furmaric acid, and the like. Preferably, the buffer or buffer system is selected from the group consisting of NaOH, citric acid, sodium dihydrogen phosphate and disodium hydrogen phosphate.

In an embodiment, the buffer is selected to adjust the pH of the S-ketamine hydrochloride pharmaceutical compositions of the present invention (e.g. the aqueous formulations described herein) into a pH in the range of from about pH 3.5 to about pH 6.5, or any amount or range therein. Preferably, the buffer is selected to adjust the pH of the S-ketamine hydrochloride compositions of the present invention to about in the range of from about pH 4.0 to about pH 5.5, or any amount or range therein, more preferably, in the range of from about pH 4.5 to about pH 5.0, or any amount or range therein.

Preferably, the concentration of the buffer and buffer system, respectively, preferably NaOH, is adjusted to provide a sufficient buffer capacity.

In an embodiment, the present invention is directed to a pharmaceutical composition comprising S-ketamine hydrochloride, water, and a buffer or buffer system, preferably NaOH; wherein the buffer or buffer system is present in an

amount sufficient to yield a formulation with a pH in the range of from about pH 4.0 to about pH 6.0, or any amount or range therein.

The pharmaceutical compositions of the present invention do not contain a preservative.

As used herein, unless otherwise noted, the terms "**antimicrobial preservative**" and "**preservative**" preferably refer to any substance that is usually added to pharmaceutical compositions in order to preserve them against microbial degradation or microbial growth. In this regard, microbial growth typically plays an essential role, i.e. the preservative serves the main purpose of avoiding microbial contamination. As a side aspect, it may also be desirable to avoid any effect of the microbes on the active ingredients and excipients, respectively, i.e. to avoid microbial degradation.

Representative examples of preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzoic acid, sodium benzoate, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorbutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, sodium propionate, thimerosal, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, isobutyl paraben, benzyl paraben, sorbic acid, and potassium sorbate.

The complete absence of preservatives in the pharmaceutical compositions of the present invention is preferred when the content of S-ketamine hydrochloride is sufficiently high so that due to its preservative property the desired shelf life or in use stability can be achieved by the presence of the drug itself. Preferably, under these circumstances the concentration of S-ketamine hydrochloride is at least eq. 120 mg/mL, preferably in the range of from about eq, 120mg/mL to about eq. 175 mg/ml, or any amount or range therein, more preferably in an amount in the range of from about eq. 125 mg/mL to about eq. 150 mg/mL, or any amount or range therein, for example at about eq. 126 mg/mL or at about eq. 140 mg/mL.

As used herein, the terms "**penetration agent**", "**penetration enhancer**", and "**penetrant**" refer to any substance that increases or facilitates absorption and / or bioavailability of the active ingredient (e.g. S-ketamine hydrochloride) of a pharmaceutical composition. Preferably, the penetration agents increases or facilitates absorption and / or bioavailability of the active ingredient (e.g. S-ketamine hydrochloride) of a pharmaceutical composition, following nasal administration (i.e. increases or facilitates absorption and / or bioavailability of the active ingredient through the mucosal membrane).

Suitable examples include, but are not limited to tetradecyl maltoside, sodium glycocholate, tauroursodeoxycholic acid (TUDCA), lecithines, and the like; and chitosan (and salts), and surface active ingredients such as benzalkonium chloride, sodium dodecyl sulfate, sodium docusate, polysorbates, laureth-9, oxtoxynol, sodium deoxycholate, polyarginine, and the like. Preferably, the penetration agent is tauroursodeoxycholic acid (TUDCA).

The penetration agent may work via any mechanism, including for example by increasing the membrane fluidity, creating transient hydrophilic pores in the epithelial cells, decreasing the viscosity of the mucus layer or opening up tight junctions. Some penetration agents (for example bile salts and fusidic acid derivatives) may also inhibit the enzymatic activity in the membrane, thereby improving bioavailability of the active ingredient.

Preferably, the penetration agent is selected to meet one or more, more preferably all, of the following general requirements:

(a) It is effective at increasing absorption (preferably nasal absorption) of the active ingredient, preferably in a temporary and / or reversible manner;

(b) It is pharmacologically inert;

(c) It is non-allergic, non-toxic and / or non-irritating;

(d) It is highly potent (effective in small amounts);

(e) It is compatible with the other components of the pharmaceutical composition;

(f) It is odorless, colorless and / or tasteless;

(g) It is accepted by regulatory agencies; and

(h) It is inexpensive and available in high purity.

In an embodiment of the present invention, the penetration agent is selected to increase penetration (absorption and / or bioavailability of the S-ketamine hydrochloride) without nasal irritation. In another embodiment of the present invention, the penetration agent is selected to improve absorption and / or biovailability of the S-ketamine hydrochloride; and further selected to enhance uniform dosing efficacy.

In an embodiment, the present invention is directed to a pharmaceutical composition comprising S-ketamine and water; herein the pharmaceutical composition does not contain an antimicrobial preservative; and wherein the pharmaceutical compositions further contains a penetration enhancer, preferably TUDCA.

In another embodiment, the present invention is directed to a pharmaceutical composition comprising S-ketamine and water; herein the pharmaceutical composition does not contain an antimicrobial preservative; and wherein the pharmaceutical compositions further contains tauroursodeoxycholic acid (TUDCA); wherein the TUDCA is present in a concentration in the range of from about 1.0 mg/mL to about 25.0 mg/mL, or any amount or range therein, preferably in a concentration in the range of from about 2.5 mg/mL to about 15 mg/mL, or any amount or range therein, preferably in a concentration in the range of from about 5 mg/mL to about 10 mg/mL, or any amount or range therein. In another embodiment, the present invention is directed to pharmaceutical composition wherein the TUDCA is present at a concentration of about 5 mg/mL. In another embodiment, the present invention is directed to pharmaceutical composition wherein the TUDCA is present at a concentration of about 10 mg/mL.

The pharmaceutical compositions of the present invention may further contain one or more additional excipients for example, wetting agents, surfactant components, solubilizing agents, thickening agents, colorant agents, antioxidant components, and the like.

Examples of a suitable antioxidant component, if used, include, but are not limited to one or more of the following: sulfites; ascorbic acid; ascorbates, such as sodium ascorbate, calcium ascorbate, or potassium ascorbate; ascorbyl palmitate; fumaric acid; ethylene diamine tetraacetic acid (EDTA) or its sodium or calcium salts; tocopherol; gallates, such as propyl gallate, octyl gallate, or dodecyl gallate; vitamin E; and mixtures thereof. The antioxidant component provides long term stability to the liquid compositions. Addition of the antioxidant component can help enhance and ensure the stability of the compositions and renders the compositions stable even after six months at 40 °C. A suitable amount of the antioxidant component, if present, is about 0.01 wt.-% to about 3 wt.-%, preferably about 0.05 wt.-% to about 2 wt.-%, of the total weight of the composition.

Solubilizing and emulsifying agents can be included to facilitate more uniform dispersion of the active ingredient or other excipient that is not generally soluble in the liquid carrier. Examples of a suitable emulsifying agent, if used, include, but are not limited to, for example, gelatin, cholesterol, acacia, tragacanth, pectin, methyl cellulose, carbomer, and mixtures thereof. Examples of a suitable solubilizing agent include polyethylene glycol, glycerin, D-mannitol, trehalose, benzyl benzoate, ethanol, trisaminomethane, cholesterol, triethanolamine, sodium carbonate, sodium citrate, sodium salicylate, sodium acetate, and mixtures thereof.

Preferably, the solubilizing agent includes glycerin. The solubilizing or emulsifying agent is/are generally present in an amount sufficient to dissolve or disperse the active ingredient, i.e. S-ketamine, in the carrier. Typical amounts when a solubilizing or an emulsifier are included are from about 1 wt.-% to about 80 wt.-%, preferably about 20 wt.-% to about 65 wt.-%, and more preferably about 25 wt.-% to about 55 wt.-%, of the total weight of the composition.

A suitable isotonizing agent, if used, includes sodium chloride, glycerin, D-mannitol, D-sorbitol, glucose, and mixtures thereof. A suitable amount of the isotonizing agent, when included, is typically about 0.01 wt.-% to about 15 wt.-%, more preferably about 0.3 wt.-% to about 4 wt.-%, and more preferably about 0.5 wt.-% to about 3 wt.-%, of the total weight of the composition.

A suspending agent or viscosity increasing agent can be added to to the pharmaceutical compositions of the present invention, to for example, increase the residence time in the nose. Suitably examples include, but are not limited to, hydroxypropyl methylcellulose, sodium carmellose, microcrystalline cellulose, carbomer, pectin, sodium alginate, chitosan salts, gellan gum, poloxamer, polyvinyl pyrrolidone, xanthan gum, and the like.

As used herein the term "**shelf life**" refers to the storage stability of a closed container of the pharmaceutical composition.

Preferably, the pharmaceutical compositions according to the present invention exhibit antimicrobial robustness that complies with the requirements for nasal pharmaceutical compositions (for example, the Ph. Eur. requirements). Requirements for the following organisms: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger (for example, A. Brasiliensis, a niger variety), and Micrococcus luteus (an in-house, J&J organism) are as listed in Table 1, below.

**Table 1: Bacterial & Fungal Requirements for Nasal Compositions**

| **Bacteria** | | | | | | |
|---|---|---|---|---|---|---|
| | **6 hrs** | **24 hrs** | **48 hrs** | **7 days** | **14 days** | **28 days** |
| **Requirement Std.** | | | | | | |
| Eur. A | - | - | log 2 | log 3 | - | NI/7d |
| Eur. B | - | - | - | - | log 3 | NI/14d |
| J&J | - | - | - | - | log 3 | NI/14d |
| U.S.P. | - | - | - | - | >= 2.0 | NI/14d |
| J.P. | - | - | - | - | >= 2.0 | NI/14d |
| **Fungi** | | | | | | |
| **Requirement Std.** | **6 hrs** | **24 hrs** | **48 hrs** | **7 days** | **14 days** | **28 days** |
| Eur. A | - | - | - | - | log 2 | NI/14d |
| Eur. B | - | - | - | - | log 1 | NI/14d |
| J.&J. | - | - | - | - | log 2.0 | NI/14d |
| U.S.P. | - | - | - | - | NI/Init. | NI/Init. |
| J.P. | - | - | - | - | NI/Init. | NI/Init. |

Preferably, antimicrobial robustness is achieved against one or more of *E. coli, S. aureus, Ps. Aeruginosa, S. spp., M. luteus and / or C. albicans.*

Preferably, the pharmaceutical compositions of the present invention exhibit a shelf-life under accelerated storage conditions of at least 1 month, more preferably at least 2 months, still more preferably at least 3 months, yet more preferably at least 4 months, most preferably at least 5 months and in particular at least 6 months. Preferably, the shelf life is determined according to Ph. Eur., particularly as described in the experimental section. Accelerated storage conditions preferably mean 40°C / 75% Relative Humidity (%RH).

Preferably, the pharmaceutical compositions of the present invention exhibit a shelf-life under ambient conditions of at least 6 months, more preferably at least 12 months, still more preferably at least 15 months, yet more preferably at least 18 months, most preferably at least 21 months and in particular at least 24 months.

In an embodiment, the present invention is directed to a pharmaceutical composition comprising: (a) S-ketamine hydrochloride 161.4 mg/mL; (b) NaOH q.s. ad pH 4.5; wherein the NaOH is preferably added to the pharmaceutical composition as a 1N solution; (c) purified water q.s. ad 1000 µL; and wherein pharmaceutical compositions does not contain antimicrobial preservative.

In another embodiment, the present invention is directed to a pharmaceutical composition comprising: (a) S-ketamine hydrochloride @ 161.4 mg/mL; (b) NaOH q.s. ad pH 4.5; wherein the NaOH is preferably added to the pharmaceutical composition as a 1N solution; (c) purified water q.s. ad 1000 µL; and (d) TUDCA @ 1.25 mg/mL; and wherein pharmaceutical compositions does not contain antimicrobial preservative.

In another embodiment, the present invention is directed to a pharmaceutical composition comprising: (a) S-ketamine hydrochloride @ 161.4 mg/mL; (b) NaOH q.s. ad pH 4.5; wherein the NaOH is preferably added to the pharmaceutical composition as a 1N solution; (c) purified water q.s. ad 1000 µL;

and (d) TUDCA @ 2.5 mg/mL; and wherein pharmaceutical compositions does not contain antimicrobial preservative.

In another embodiment, the present invention is directed to a pharmaceutical composition comprising: (a) S-ketamine hydrochloride @ 161.4 mg/mL; (b) NaOH q.s. ad pH 4.5; wherein the NaOH is preferably added to the pharmaceutical composition as a 1N solution; (c) purified water q.s. ad 1000 µL; and (d) TUDCA @ 5 mg/mL; and wherein pharmaceutical compositions does not contain antimicrobial preservative.

In another embodiment, the present invention is directed to a pharmaceutical composition comprising: (a) S-ketamine hydrochloride @ 161.4 mg/mL; (b) NaOH q.s. ad pH 4.5; wherein the NaOH is preferably added to the pharmaceutical composition as a 1N solution; (c) purified water q.s. ad 1000 µL; and (d) TUDCA @ 10 mg/mL; and wherein pharmaceutical compositions does not contain antimicrobial preservative.

In another embodiment, the present invention is directed to a pharmaceutical composition comprising: (a) S-ketamine hydrochloride @ 161.4 mg/mL; (b) NaOH q.s. ad pH 4.5; wherein the NaOH is preferably added to the pharmaceutical composition as a 1N solution; (c) purified water q.s. ad 1000 µL; and (d) TUDCA @ 15 mg/mL; and wherein pharmaceutical compositions does not contain antimicrobial preservative.

In an embodiment, the pharmaceutical composition of the present invention is prepared by adding water to the S-ketamine hydrochloride; followed by addition of 1N $NaOH_{(aq)}$ to adjust the pH of the resulting mixture to the desired pH, preferably to a pH in the range of from about pH 3.5 to about pH 6.0, more preferably to about pH in the range of from about pH 4.0 to about pH 5.0, more preferably to about pH 4.5.

In a preferred embodiment, the pharmaceutical compositions of the present invention are ready to use, i.e. do not require particular treatment steps such as dissolution in a solvent before they may be administered to the patient.

A skilled person recognizes that the pharmaceutical compositions of the present invention may alternatively be commercialized as a precursor in form of a dry powder that is to be dissolved or dispersed in an appropriate amount of water prior to the first use.

A further aspect of the invention relates to a pharmaceutical dosage form comprising the pharmaceutical composition according to the invention. All preferred embodiments that are described above in connection with the composition according to the invention also apply to the dosage form according to the invention.

In an embodiment, the dosage form according to the invention is adapted for nasal administration. Preferably, the dosage form according to the invention is adapted for administration once every couple of days, on individually determined days only; or once daily, twice daily, thrice daily, four times daily, five times daily, six times daily or even more frequently; or clustered as between 2 and up to 8 consecutive administrations within a limited time period ranging from 1 to about 60 minutes.

The present invention is further directed to methods for the treatment of depression, preferably resistant depression or treatment refractory depression, comprising administering to a subject in need thereof, a therapeutically effective amount of any of the pharmaceutical compositions a described herein. Preferably, the administration is nasal.

In an embodiment, the present invention is directed to a method for the treatment of depression, preferably resistant depression or treatment refractory depression, comprising the nasal administration of the pharmaceutical composition according to the invention as described above or of the pharmaceutical dosage form according to the invention as described above, to a subject in need thereof.

Major Depressive Disorder is defined as the presence of one of more major depressive episodes that are not better accounted for psychotic disorder or bipolar

disorder. A major depressive episode is characterized by meeting five or more of the following criteria during the same 2 week period which represent a change in functioning and include at least depressed/ sad mood or loss of interest and pleasure, indifference or apathy, or irritability and is usually associated with a change in a number of neurovegetative functions, including sleep patterns, appetite and body weight, motor agitation or retardation, fatigue, impairment in concentration and decision making, feelings of shame or guilt, and thoughts of death or dying (*Harrison's Principles of Internal Medicine*, 2000). Symptoms of a depressive episode include depressed mood; markedly diminished interest or pleasure in all, or almost all, activities most of the day; weight loss when not dieting or weight gain, or decrease or increase in appetite nearly every day; insomnia or hypersomnia nearly every day; psychomotor agitation or retardation nearly every day; fatigue or loss of energy nearly every day; feelings of worthlessness or excessive or inappropriate guilt nearly every day; diminished ability to think or concentrate, or indecisiveness, nearly every day; recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide. Further, the symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning. (*Diagnostic and Statistical Manual of Mental Disorders*, 4$^{th}$ Edition, American Psychiatric Association, 1994).

As used herein, the term "**depression**" shall be defined to include major depressive disorder, unipolar depression, treatment-refractory depression, resistant depression, anxious depression, bipolar depression and dysthymia (also referred to as dysthymic disorder). Preferably, the depression is major depressive disorder, unipolar depression, treatment-refractory depression, resistant depression, anxious depression or bipolar depression.

As used herein, the term "**treatment-refractory or treatment-resistant depression**" and the abbreviation "**TRD**" shall be defined as major depressive disorder that does not respond to adequate courses of at least two antidepressants, preferably two or more antidepressants, more preferably two to three, antidepressants.

As used herein, the term "**bipolar depression**" is intended to mean the depression associated with, characteristic of or symptomatic of a bipolar disorder. Thus, methods of treating bipolar depression of the present invention are directed to methods which treat the depression and / or depressed phase of bipolar disorders.

One skilled in the art will recognize that the failure to respond to an adequate course of a given antidepressant may be determined retrospectively or prospectively. In an embodiment, at least one of the failures to respond to an adequate course of antidepressant is determined prospectively. In another embodiment, at least two of the failures to respond to an adequate course of antidepressant are determined prospectively. In another embodiment, at least one of the failures to respond to an adequate course of antidepressant is determined retrospectively. In another embodiment, at least two of the failures to respond to an adequate course of antidepressant are determined retrospectively.

As used herein, unless otherwise noted, the terms "**treating**", "**treatment**" and the like, shall include the management and care of a subject or patient (preferably mammal, more preferably human) for the purpose of combating a disease, condition, or disorder and includes the administration of a compound of the present invention to prevent the onset of the symptoms or complications, alleviate the symptoms or complications, or eliminate the disease, condition, or disorder.

The term "**therapeutically effective amount**" as used herein, means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

Optimal dosages to be administered may be readily determined by those skilled in the art, and will vary with the particular compound used, the mode of administration, the strength of the preparation, the mode of administration, the number of consecutive administrations within a limited period of time (e.g. up to 60

minutes) and the advancement of the disease condition. In addition, factors associated with the particular patient being treated, including patient age, weight, diet and time of administration, will result in the need to adjust dosages.

The term "**subject**" as used herein, refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment. Preferably, the subject has experienced and / or exhibited at least one symptom of the disease or disorder to be treated and / or prevented.

To provide a more concise description, some of the quantitative expressions herein are recited as a range from about amount X to about amount Y. It is understood that wherein a range is recited, the range is not limited to the recited upper and lower bounds, but rather includes the full range from about amount X through about amount Y, or any amount or range therein.

To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "**about**". It is understood that whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including approximations due to the experimental and/or measurement conditions for such given value.

The following Examples are set forth to aid in the understanding of the invention, and are not intended and should not be construed to limit in any way the invention set forth in the claims which follow thereafter.

**Example 1: Microbial Challenge Test for Nasal Spray Pharmaceutical Composition Containing S-ketamine Hydrochloride (eq. 140 mg/ml)**

An aqueous formulation of S-ketamine hydrochloride referred to as "S-ketamine eq. 140 mg/ml below, was prepared by mixing S-ketamine hydrochloride (at a concentration of 161.4 mg/ml) in water and then adding 1N $NaOH_{(aq)}$ to pH 5.0.

A challenge test was initiated to investigate whether the formulation could prevent microorganisms from proliferating. The challenge test consisted of challenging the formulation with a prescribed inoculum of microorganisms. The inoculated formulation was then stored at room temperature and at specified time intervals a sample was withdrawn to count the microorganisms in the sample.

As shown in Table 2 below, the challenge test results on S-ketamine eq. 140 mg/ml pH 5.0 show that S-ketamine eq. 140mg/ml pH 5.0 reduced the original spike ($10^5$-$10^6$ CFU/ml) of bacteria (i.e. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and environmental isolate *Staphylococcus haemolyticus*) and also of the yeast *Candida albicans*. S-ketamine eq. 140mg/ml pH 5.0 was not able to reduce the original spike of *Aspergillus brasiliensis* to the same extent as for the bacteria and yeast, but no increase was observed after 28 days of incubation at room temperature.

**Table 2: Challenge Test S-ketamine eq. 140 mg/ml pH 5.0 (CFU/ml)**

| Organism | Blank at 0 hours | Product | | | | |
|---|---|---|---|---|---|---|
| | | at 0 hours | at 2 days | at 7 days | at 14 days | at 28 days |
| *A. brasiliensis* | 1.50 x $10^5$ | 1.90 x $10^5$ | ND | 4.80 x $10^4$ | 5.80 x $10^4$ | 2.65 x $10^4$ |
| *C. albicans* | 1.15 x $10^5$ | 6.50 x $10^4$ | ND | < 5 | < 5 | < 5 |
| *P. aeruginosa* | 2.65 x $10^5$ | 1.59 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *S. aureus* | 1.90 x $10^5$ | 1.70 x $10^5$ | < 50 | < 5 | < 5 | < 5 |
| *E. coli* | 1.20 x $10^5$ | 6.00 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *S. haemolyticus* | 1.20 x $10^5$ | 7.50 x $10^4$ | < 50 | < 5 | < 5 | < 5 |

ND: not determined

## Example 2: Microbial Challenge for Nasal Spray Pharmaceutical Composition Containing S-ketamine Hydrochloride

Aqueous formulation of S-ketamine hydrochloride as listed in Table 3, below, were prepared by mixing S-ketamine hydrochloride (at the listed concentrations) in water and then adding 1N $NaOH_{(aq)}$ to the listed pH levels. Formulation F-5 additionally contained 10 mg/mL of tauroursodeoxycholic acid (TUDCA).

**Table 3: Composition of edge of failure batches**

| Formulation | Concentration S-Ketamine HCl (mg/ml) | pH |
|---|---|---|
| F-1 | eq. 126 | 4.0 |
| F-2 | eq. 140 | 4.5 |
| F-3 | eq. 126 | 5.0 |
| F-4 | eq. 126 | 4.5 |
| F-5 | eq. 140 | 4.5 |

* This formulation also contained 10 mg/mL Tauroursodeoxycholic acid (TUDCA)

Low Level *A brasiliensis* Challenge

The formulations listed in Table 3, above were subjected to a low level challenge with *A. brasiliensis* to evaluate whether the formulations would be able to reduce this lower spike, with results as shown in Table 4, below. A spike of $10^3$ CFU/ml was chosen instead of $10^5$-$10^6$ CFU/ml, which is the standard spike for a challenge test.

**Table 4: Low Level Challenge with *A. brasiliensis***

| | Product (CFU/ml) | | | |
|---|---|---|---|---|
| **Formulation** | **0 hours** | **7 days** | **14 days** | **28 days** |
| F-1 | 4.45 E+02 | 2.35 E+02 | 2.15 E+02 | 1.75 E+02 |
| F-2 | 3.80 E+02 | 2.50 E+02 | 2.00 E+02 | 2.00 E+02 |
| F-3 | 5.50 E+02 | 2.75 E+02 | 2.65 E+02 | 1.10 E+02 |
| F-4 | 3.85 E+02 | 2.70 E+02 | 2.60 E+02 | 1.55 E+02 |
| F-5 | 4.10 E+02 | 4.00 E+02 | 3.15 E+02 | 2.25 E+02 |
| Blank | 4.75 E+02 | NA | NA | NA |

The results, as listed in Table 3, above, show that in none of the tested formulation was an increase in *Aspergillus brasiliensis* observed after 28 days of incubation, rather a minor and slow decrease was observed.

Full AET Challenge Test

The formulations listed in Table 3 were additionally subjected to a Full AET challenge, with results for the individual formulations are shown in Tables 5-9, below. Additionally, Table 10 below, provides results for a Full AET challenge of a reference formulation containing 0.00 mg/mL S-ketamine hydrochloride, denatonium benzoate (to mimic the taste of the S-ketamine HCl formulation(s)), and adjusted to pH 5.21 with 1N NaOH.

**Table 5: Full AET Challenge Test**

**Formulation F-1: S-ketamine eq. 126 mg/ml pH 4.0 (CFU/ml)**

| Organism | Blank at 0 hrs | Product | | | | |
|---|---|---|---|---|---|---|
| | | at 0 hrs | at 2 days | at 7 days | at 14 days | at 28 days |
| *A. brasiliensis* | $1.55 \times 10^5$ | $2.15 \times 10^5$ | ND | $5.30 \times 10^4$ | $2.10 \times 10^4$ | $3.5 \times 10^3$ |
| *C. albicans* | $1.05 \times 10^5$ | $1.15 \times 10^4$ | ND | < 5 | < 5 | < 5 |
| *P. aeruginosa* | $5.05 \times 10^5$ | $1.10 \times 10^4$ | < 50 | < 5 | < 5 | < 5 |
| *S. aureus* | $6.00 \times 10^5$ | $6.25 \times 10^5$ | < 50 | < 5 | < 5 | < 5 |
| *E. coli* | $6.05 \times 10^5$ | $4.00 \times 10^4$ | < 50 | < 5 | < 5 | < 5 |
| *M. luteus* | $1.65 \times 10^5$ | $7.50 \times 10^4$ | < 50 | < 5 | < 5 | < 5 |

ND: not determined

**Table 6: Full AET Challenge Test**

**Formulation F-2: S-ketamine eq. 140 mg/ml pH 4.5 (CFU/ml)**

| Organism | Blank at 0 hrs | Product | | | | |
|---|---|---|---|---|---|---|
| | | at 0 hrs | at 2 days | at 7 days | at 14 days | at 28 days |

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. brasiliensis* | 1.55 x $10^5$ | 1.20 x $10^5$ | ND | 1.65 x $10^4$ | 1.00 x $10^4$ | 2.0 x $10^3$ |
| *C. albicans* | 1.05 x $10^5$ | 1.15 x $10^4$ | ND | < 5 | < 5 | < 5 |
| *P. aeruginosa* | 5.05 x $10^5$ | 7.55 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *S. aureus* | 6.00 x $10^5$ | 5.70 x $10^5$ | < 50 | < 5 | < 5 | < 5 |
| *E. coli* | 6.05 x $10^5$ | 2.80 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *M. luteus* | 1.65 x $10^5$ | 1.70 x $10^4$ | < 50 | < 5 | < 5 | < 5 |

ND: not determined

**Table 7: Full AET Challenge Test**

**Formulation F-3: S-ketamine eq. 126 mg/ml pH 5.0 (CFU/ml)**

| **Organism** | **Blank at 0 hrs** | **Product** | | | | |
|---|---|---|---|---|---|---|
| | | **at 0 hrs** | **at 2 days** | **at 7 days** | **at 14 days** | **at 28 days** |
| *A. brasiliensis* | 1.55 x $10^5$ | 1.90 x $10^5$ | ND | 3.50 x $10^4$ | 2.15 x $10^4$ | 8.0 x $10^3$ |
| *C. albicans* | 1.05 x $10^5$ | 1.60 x $10^4$ | ND | < 5 | < 5 | < 5 |
| *P. aeruginosa* | 5.05 x $10^5$ | 1.03 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *S. aureus* | 6.00 x $10^5$ | 6.05 x $10^5$ | < 50 | < 5 | < 5 | < 5 |
| *E. coli* | 6.05 x $10^5$ | 2.00 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *M. luteus* | 1.65 x $10^5$ | 1.60 x $10^4$ | < 50 | < 5 | < 5 | < 5 |

5 ND: not determined

**Table 8: Full AET Challenge Test**

**Formulation F-4: S-ketamine eq. 126 mg/ml pH 4.5 (CFU/ml)**

| **Organism** | **Blank at 0 hrs** | **Product** | | | | |
|---|---|---|---|---|---|---|
| | | **at 0 hrs** | **at 2 days** | **at 7 days** | **at 14 days** | **at 28 days** |
| *A. brasiliensis* | 1.55 x $10^5$ | 2.10 x $10^5$ | ND | 5.30 x $10^4$ | 2.10 x $10^4$ | 5.5 x $10^3$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. albicans* | 1.05 x $10^5$ | 2.85 x $10^4$ | ND | < 5 | < 5 | < 5 |
| *P. aeruginosa* | 5.05 x $10^5$ | 8.35 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *S. aureus* | 6.00 x $10^5$ | 7.60 x $10^5$ | < 50 | < 5 | < 5 | < 5 |
| *E. coli* | 6.05 x $10^5$ | 4.80 x $10^4$ | < 50 | < 5 | < 5 | < 5 |
| *M. luteus* | 1.65 x $10^5$ | 7.00 x $10^4$ | < 50 | < 5 | < 5 | < 5 |

ND: not determined

**Table 9: Full AET Challenge Test**

**Formulation F-5: S-ketamine eq. 140 mg/ml pH 4.5 , TUDCA @ 10 mg/mL**

**(CFU/ml)**

| Organism | Blank at 0 hrs | Product | | | | |
|---|---|---|---|---|---|---|
| | | at 0 hrs | at 2 days | at 7 days | at 14 days | at 28 days |
| *A. brasiliensis* | 1.55 x $10^5$ | 8.50 x $10^5$ | ND | 4.15 x $10^4$ | 2.40 x $10^4$ | 8.5 x $10^3$ |
| *C. albicans* | 1.05 x $10^5$ | 1.30 x $10^4$ | ND | < 5 | < 5 | < 5 |
| *P. aeruginosa* | 5.05 x $10^5$ | < 50 | < 50 | < 5 | < 5 | < 5 |
| *S. aureus* | 6.00 x $10^5$ | 6.20 x $10^5$ | < 50 | < 5 | < 5 | < 5 |
| *E. coli* | 6.05 x $10^5$ | < 50 | < 50 | < 5 | < 5 | < 5 |
| *M. luteus* | 1.65 x $10^5$ | 6.50 x $10^4$ | < 50 | < 5 | < 5 | < 5 |

ND: not determined

**Table 10: Full AET Challenge Test**

**Reference Formulation: 0.0 mg/mL S-ketamine, Denatonium Benzoate (to mimic S-ketamine HCl taste), pH 5.21**

| Organism | Blank at 0 hours | At 0 hours | At 2 days | At 7 days | At 14 days | At 28 days |
|---|---|---|---|---|---|---|
| *A. Brasileinsis* | 1.55 x $10^5$ | 1.75 x $10^5$ | - | 4.35 x $10^4$ | 8.00 x $10^4$ | 9.00 x $10^4$ |
| *C. Albicans* | 1.05 x $10^5$ | 1.20 x $10^5$ | - | 1.02 x $10^5$ | 1.40 x $10^5$ | 1.30 x $10^5$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| *P. aeruginosa* | $5.05 \times 10^5$ | $4.05 \times 10^5$ | $6.55 \times 10^5$ | $>2.00 \times 10^5$ | $9.75 \times 10^5$ | $2.40 \times 10^6$ |
| *S. aureus* | $6.00 \times 10^5$ | $5.15 \times 10^5$ | $3.90 \times 10^5$ | $1.55 \times 10^2$ | <5 | <5 |
| *E. coli* | $6.05 \times 10^5$ | $5.55 \times 10^5$ | $6.40 \times 10^5$ | $>2.00 \times 10^5$ | $8.25 \times 10^5$ | $1.09 \times 10^6$ |
| *M. luteus* | $1.65 \times 10^5$ | $6.00 \times 10^4$ | $8.50 \times 10^4$ | $4.30 \times 10^2$ | $2.50 \times 10^3$ | $3.90 \times 10^2$ |

As shown in Tables 5-10 above, after 28 days, all the tested formulations reduced the original spike ($10^5$-$10^6$ CFU/ml) of bacteria (i.e. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and environmental isolate *Staphylococcus haemolyticus*) and environmental *Micrococcus Luteus*, and also of the yeast *Candida albicans*. The tested formulations were not able to reduce the original spike of *Aspergillus brasiliensis* to the same extent as for the bacteria and yeast, but no increase was observed after 28 days of incubation at room temperature.

In summary, the results presented in Biological Example 1 and Biological Example 2 indicated that S-ketamine hydrochloride exhibits strong antimicrobial properties against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli,* environmental isolate *Staphylococcus haemolyticus,* environmental *Micrococcus Luteus*, and the yeast *Candida albicans*. For *Aspergillus brasiliensis* the decrease in microorganisms is less pronounced, but no increase was observed, indicating some inhibitory activity.

## Example 3: Rat PK Study

Rat Study Procedure:

Male Sprague Dawley rats weighing around 250 g were housed individually in cages of 800 cm² with limited access under routine test conditions of temperature (20°C-24°C), relative humidity (45%-65%) and 12/12 light cycle. Nine male Sprague Dawley rats per formulation were tested. The animals were shortly anesthetized with isoflurane (2-2.5% for 5 minutes) and were then dosed intranasally. The test formulations were dosed at a volume of 10 µl/rat in a single

dose. The approximate body weight of the rats was 250 g, thus S-ketamine was dosed at ± 6 mg/kg. Intranasal dosing was done by placing a small pipette at the opening of the right nostril, then the compound was given and the rat was kept for another minute under anesthesia. Afterwards, the rat was placed back in his cage. Blood for pharmacokinetics was taken at 2.5, 5, 10 and 15 minutes after dosing via the tail vein.

Formulation 3-A:

S-ketamine hydrochloride (eq. 150 mg/mL) was mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.52.

Formulations 3-B, 3-C, 3-D:

S-ketamine hydrochloride (eq. 150 mg/mL) and TUDCA (3-B:@1.25 mg/mL, 3-C: @5.0 mg/mL, 3-D: @10 mg/mL) were mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.51.

Formulation 3-E:

S-ketamine hydrochloride (eq. 150 mg/mL) was mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 3.77.

Formulation 3-F:

S-ketamine hydrochloride (eq. 150 mg/mL) was mixed with water, then citric acid monohydrate at 2.73 mg/ml and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.45.

Formulations 3-A through 3-F were tested according to the procedure as described above, with measured plasma levels of S-ketamine as listed in Table 11, below.

**Table 11: Rat pK Study Results**

| | Plasma Level Mean ± SEM (ng/mL) | | | |
|---|---|---|---|---|
| **Formulations** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 3-A | 150 ± 24 | 254 ± 24 | 374 ± 26 | 344 ± 29 |
| 3-B | 135 ± 16 | 288 ± 24 | 379 ± 102 | 353 ± 37 |
| 3-C | 246 ± 28 | 499 ± 52 | 522 ± 47 | 446 ± 35 |
| 3-D | 283 ± 24 | 474 ± 41 | 430 ± 32 | 338 ± 28 |

| | | | | |
|---|---|---|---|---|
| 3-E | 145 ± 24 | 240 ± 24 | 296 ± 22 | 277 ± 29 |
| 3-F | 144 ± 22 | 217 ± 20 | 292 ± 31 | 284 ± 36 |

## Example 4: Dog PK Study

Beagle dogs weighing from 7 to 12 kg were used in this study. The dogs were housed separately in barriered testing rooms with limited access, and placed in individual pens under routine test conditions of temperature (18°C-25°C), ventilation (10-15 cycles/hour) and illumination.

Four beagle dogs per formulation were tested, 2 males and 2 females per group. The test formulations were dosed at 3 mg/kg, a volume of 20 µl/kg in a single dose was given. The animals were dosed intranasally by placing a small pipette at the opening of the right nostril. For the next minute, the head of the dog was tilted so no fluid could run out of the nose or into the throat. Afterwards, the dog was placed back in his pen.

Blood for pharmacokinetics were taken at 5, 10, 15 and 30 minutes after dosing via the jugular vein.

Formulation 4-A:

S-ketamine hydrochloride (eq. 150 mg/mL) was mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.52.

Formulation 4-B:

S-ketamine hydrochloride (eq. 150 mg/mL) and TUDCA (10 mg/mL) were mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.51.

Formulations 4-A and 4-B were tested according to the procedure as described above, with measured plasma levels of S-ketamine as listed in Table 12, below.

**Table 12: Dog pK Study Results**

| | Plasma Level Mean ± SEM (ng/mL) | |
|---|---|---|
| **Time (min)** | **Formulation 4-A** | **Formulation 4-B** |
| 5 | 613 ± 206 | 3296 ±1901 |

| | | |
|---|---|---|
| 10 | 681 ± 265 | 1728 ±203 |
| 15 | 380 ± 66 | 10556 ±397 |
| 30 | 307 ± 69 | 192 ±40 |

## Example 5: Rat PK Study

Rat Study Procedure:

Male Sprague Dawley rats weighing around 250 g were housed individually in cages of 800 cm² with limited access under routine test conditions of temperature (20°C-24°C), relative humidity (45%-65%) and 12/12 light cycle. Nine male Sprague Dawley rats per formulation were tested. The animals were shortly anesthetized with isoflurane (2-2.5% for 5 minutes) and were then dosed intranasally. The test formulations were dosed at a volume of 10 µl/rat or 25 µl/rat (as noted below) in a single dose. The approximate body weight of the rats was 250 g, thus S-ketamine was dosed at ± 6 mg/kg. Intranasal dosing was done by placing a small pipette at the opening of the right nostril, then the compound was given and the rat was kept for another minute under anesthesia. Afterwards, the rat was placed back in his cage. Blood for pharmacokinetics was taken at 2.5, 5, 10 and 15 minutes after dosing via the tail vein.

Formulation 5-A:

S-ketamine hydrochloride (eq. 150 mg/mL) was mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.5.

Formulations 5-B, 5-C, 5-D:

S-ketamine hydrochloride (eq. 150 mg/mL) and TUDCA (5-B:@5 mg/mL, 5-C: @10.0 mg/mL, 5-D: @15 mg/mL) were mixed with water and the pH of the resulting mixture adjusted with 1N NaOH to pH 4.5.

Reference Formulation 5-E:

TUDCA @ 10 mg/mL

Formulations 5-A through 5-D and reference formulation 5-E were tested according to the procedure as described above, with measured S-ketamine and TUDCA plasma levels, as listed in Table 13, below.

**Table 13: Rat PK Study Results**

| | S-Ketamine Plasma Level Mean ± SEM (ng/mL) Dosing 10 µl/rat | | | |
|---|---|---|---|---|
| **Formulations** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 5-A | 130 ± 12 | 271± 19 | 367 ± 33 | 352 ± 37 |
| 5-B | 365 ± 76 | 511 ± 53 | 525 ± 71 | 430 ± 60 |
| 5-C | 494 ± 62 | 681 ± 60 | 623 ± 65 | 492 ± 60 |
| 5-D | 422 ± 53 | 588 ± 78 | 556 ± 72 | 432 ± 51 |
| | **S-Ketamine Plasma Level Mean ± SEM (ng/mL) Dosing 25 µl/rat** | | | |
| **Formulations** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 5-A | 339 ± 109 | 642 ± 143 | 899 ± 118 | 797 ± 143 |
| 5-C | 732 ± 246 | 1154 ± 304 | 1018 ± 261 | 997 ± 183 |
| | **TUDCA Plasma Level Mean ± SEM (ng/mL) Dosing 25 µl/rat** | | | |
| **Formulations** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 5-C | 250 ± 17 | 346 ± 27 | 303 ± 35 | 242 ± 32 |
| 5-E | 624 ± 128 | 1209 ± 219 | 822 ± 152 | 756 ±118 |

Nasal Cavity Histopathology

Nasal cavity histology was evaluated microscopically and scored for tissue damage, including but not limited to degeneration and detachment, as follows: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe/massive.

Rats treated with Formulations 5-A through 5-D at 10 µL/ rat and rats treated with Formulation 5-A, 5-C and reference formulation 5-E at 25 µL/rat were evaluated and scored as described above, with results as listed in Table 14, below.

**Table 14: Nasal cavity (Respiratory Epithelium) Histopathology**

| Formulations dosed at 10 µl/rat | |
|---|---|
| 5-A | Minimal degeneration of respiratory epithelium; No tissue change |
| 5-B and 5-C | Slight to moderate degeneration of respiratory epithelium, with / without detachment of respiratory epithelium |

| | |
|---|---|
| 5-D | Slight degeneration of respiratory epithelium, with detachment of respiratory epithelium |
| **Formulations dosed at 25 µl/rat** | |
| 5-A | Slight degeneration of respiratory epithelium; slightly more severe than minimal scope |
| 5-C | Minimal to moderate degeneration of respiratory epithelium; more variable than Formulation 5-A |
| 5-E (Ref.) | No degeneration |

## Example 6: Rat PK Study

Rat Study Procedure:

Male Sprague Dawley rats weighing around 225 g were housed individually in cages of 800 cm² with limited access under routine test conditions of temperature (20°C-24°C), relative humidity (45%-65%) and 12/12 light cycle. Nine male Sprague Dawley rats per formulation were tested. The animals were shortly anesthetized with isoflurane (2-2.5% for 5 minutes) and were then dosed intranasally. The test formulations were dosed at a volume of 10 µl/rat at a dose of 1.4 mg/rat, in a single dose. Intranasal dosing was done by placing a small pipette at the opening of the right nostril, then the compound was given and the rat was kept for another minute under anesthesia. Afterwards, the rats were placed back in their cage. Blood for pharmacokinetics was taken at 2.5, 5, 10 and 15 minutes after dosing via the tail vein. The nine intranasal formulations comprising S-ketamine that were tested according to the above procedure, were as listed in Table 15 below.

**Table 15: Rat PK Study Formulations Tested**

**S-Ketamine HCl (eq.) 140 mg/mL Nasal Solution**

| ID No. | S-Ketamine HCl (eq.) | TUDCA | Citric Acid | Other Formulation Component(s) |
|---|---|---|---|---|
| 6-A | 140mg/ml | 6 mg/ml | | |
| 6-B | 140mg/ml | | 1.5mg/ml | 10mg/ml NaCMC 30-40 mPa.s |
| 6-C | 140mg/ml | | 1.5mg/ml | 10mg/ml HPMC 5 mPa.s |
| 6-D | 140mg/ml | | 1.5mg/ml | 100mg/ml PEG 4000 |

| | | | | |
|---|---|---|---|---|
| 6-E | 140mg/ml | | 1.5mg/ml | 100mg/ml PEG 10000 |
| 6-F | 140mg/ml | 6 mg/ml | 1.5mg/ml | 10mg/ml NaCMC 30-40 mPa.s |
| 6-G | 140mg/ml | 6 mg/ml | 1.5mg/ml | 10mg/ml HPMC 5 mPa.s |
| 6-H | 140mg/ml | 6 mg/ml | 1.5mg/ml | 100mg/ml PEG 4000 |
| 6-I | 140mg/ml | 6 mg/ml | 1.5mg/ml | 100mg/ml PEG 10000 |

Formulations 6-A through 6-I were tested according to the procedure as described above, with average plasma levels of S-ketamine (in ng/mL) as a function of time, with values for each formulation and animal tested, as listed in Table 16, below.

**Table 16: S-ketamine Plasma Concentration (ng/ml)**

| **Formulation 6-A** | | | | |
|---|---|---|---|---|
| **Animal** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 1 | 596 | 588 | 615 | 506 |
| 2 | 370 | 496 | 527 | 368 |
| 3 | 539 | 662 | 535 | 405 |
| 4 | 234 | 535 | 548 | 416 |
| 5 | 591 | 774 | 608 | 461 |
| 6 | 539 | 680 | 610 | 444 |
| 7 | 514 | 1130 | 1370 | 1080 |
| 8 | 448 | 813 | 648 | 495 |
| 9 | 672 | 975 | 798 | 666 |
| **Formulation 6-B** | | | | |
| **Animal** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 1 | **95.9** | **176** | **266** | **231** |
| 2 | **85.4** | **172** | **266** | **231** |
| 3 | **60.9** | **117** | **184** | **192** |
| 4 | **110** | **177** | **221** | **211** |
| 5 | **141** | **201** | **231** | **200** |
| 6 | **214** | **361** | **439** | **398** |
| 7 | **180** | **274** | **393** | **438** |
| 8 | **210** | **262** | **369** | **384** |
| 9 | **196** | **251** | **284** | **288** |
| **Formulation 6-C** | | | | |
| **Animal** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 1 | **126** | **229** | **276** | **257** |
| 2 | **155** | **201** | **225** | **216** |
| 3 | **80.7** | **148** | **240** | **229** |
| 4 | **109** | **184** | **282** | **308** |

| | | | | |
|---|---|---|---|---|
| 5 | 71.2 | 196 | 334 | 344 |
| 6 | 194 | 239 | 340 | 393 |
| 7 | 181 | 329 | 511 | 475 |
| 8 | 95.3 | 145 | 202 | 202 |
| 9 | 254 | 400 | 631 | 661 |
| Formulation 6-D | | | | |
| Animal | 2.5 min | 5 min | 10 min | 15 min |
| 1 | 151 | 268 | 406 | 377 |
| 2 | 55.3 | 95.5 | 150 | 149 |
| 3 | 83..2 | 215 | 259 | 296 |
| 4 | 234 | 341 | 538 | 539 |
| 5 | 230 | 343 | 422 | 396 |
| 6 | 120 | 169 | 225 | 217 |
| 7 | 169 | 303 | 481 | 441 |
| 8 | 101 | 165 | 261 | 253 |
| 9 | 207 | 327 | 527 | 601 |
| Formulation 6-E | | | | |
| Animal | 2.5 min | 5 min | 10 min | 15 min |
| 1 | 93.3 | 189 | 232 | 314 |
| 2 | 299 | 363 | 476 | 512 |
| 3 | 90.4 | 208 | 209 | 172 |
| 4 | 176 | 229 | 236 | 230 |
| 5 | 177 | 257 | 357 | 345 |
| 6 | 253 | 360 | 372 | 347 |
| 7 | 169 | 228 | 267 | 240 |
| 8 | 142 | 231 | 353 | 399 |
| 9 | 130 | 184 | 253 | 224 |
| Formulation 6-F | | | | |
| Animal | 2.5 min | 5 min | 10 min | 15 min |
| 1 | 307 | 654 | 588 | 516 |
| 2 | 534 | 1060 | 918 | 741 |
| 3 | 450 | 657 | 567 | 412 |
| 4 | 213 | 511 | 394 | 328 |
| 5 | 490 | 561 | 474 | 409 |
| 6 | 607 | 946 | 827 | 658 |
| 7 | 381 | 568 | 494 | 407 |
| 8 | 1440 | 881 | 576 | 495 |
| 9 | 653 | 796 | 639 | 518 |
| Formulation 6-G | | | | |
| Animal | 2.5 min | 5 min | 10 min | 15 min |
| 1 | 96.8 | 268 | 231 | 191 |
| 2 | 477 | 699 | 648 | 667 |
| 3 | 271 | 382 | 374 | 306 |
| 4 | 230 | 464 | 355 | 315 |

| | | | | |
|---|---|---|---|---|
| 5 | **651** | **841** | **684** | **564** |
| 6 | **164** | **389** | **519** | **421** |
| 7 | **234** | **429** | **542** | **441** |
| 8 | **399** | **933** | **718** | **547** |
| 9 | **322** | **510** | **446** | **378** |
| **Formulation 6-H** | | | | |
| **Animal** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 1 | **232** | **306** | **322** | **261** |
| 2 | **336** | **544** | **736** | **599** |
| 3 | **287** | **658** | **621** | **619** |
| 4 | **349** | **586** | **525** | **587** |
| 5 | **253** | **541** | **494** | **463** |
| 6 | **315** | **580** | **607** | **591** |
| 7 | **80.6** | **162** | **181** | **136** |
| 8 | **303** | **452** | **369** | **259** |
| 9 | **231** | **299** | **273** | **232** |
| **Formulation 6-I** | | | | |
| **Animal** | **2.5 min** | **5 min** | **10 min** | **15 min** |
| 1 | **247** | **377** | **402** | **354** |
| 2 | **53.9** | **302** | **464** | **436** |
| 3 | **229** | **382** | **383** | **331** |
| 4 | **185** | **264** | **286** | **256** |
| 5 | **160** | **155** | **293** | **241** |
| 6 | **658** | **482** | **320** | **343** |
| 7 | **312** | **482** | **381** | **298** |
| 8 | **380** | **424** | **495** | **604** |
| 9 | **207** | **412** | **537** | **488** |

Nasal Cavity Histopathology

Nasal cavity histology was evaluated microscopically and scored for tissue damage. The nose was sampled, fixed in 10% formalin prior to evaluation. Rats treated with Formulations 6-A, 6-B and 6-F were evaluated, with results as noted below.

Formulation 6-A: Minimal to slight degeneration of the respiratory epithelium in all rats; minimal edema / hemorrhage in the submucosa of olfactory epithelium / ethmoid turbinate in 4 of 9 rats.

Formulation 6-B: Minimal edema in the submucosa of the ethmoid turbinates in 2 of 9 rats.

Formulation 6-F: Minimal to slight degeneration of the respiratory epithelium in all rats; minimal degeneration of the olfactory epithelium in 5 of 9 rats; minimal edema / hemorrhage in the submucosa of olfactory / ethmoid turbinates in 5 of 9 rats.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations and/or modifications as come within the scope of the following claims and their equivalents.

## CLAIMS

1. A pharmaceutical composition comprising S-ketamine hydrochloride and water; wherein the pharmaceutical composition does not contain an antimicrobial preservative, and wherein the composition further contains a penetrating agent which is tauroursodeoxycholic acid.

2. The pharmaceutical composition according to Claim 1, which

(i) additionally contains a buffer; and/or

(ii) has a pH value within the range of from 4.0 to 6.5.

3. The pharmaceutical composition according to Claim 1, wherein the S-ketamine hydrochloride is present in a concentration in the range of eq. 100 mg/mL to eq. 200 mg/mL, based on the total volume of the composition; or wherein the S-ketamine hydrochloride is present in a concentration in the range of eq. 125 mg/mL to eq. 150 mg/mL, based on the total volume of the composition; or
wherein the S-ketamine hydrochloride is present in a concentration in the range of 126 mg / mL to 162 mg/mL, based on the total volume of the composition.

4. The pharmaceutical composition according to Claim 1, wherein the pharmaceutical composition is formulated for nasal administration.

5. The pharmaceutical composition according to Claim 1, wherein the pharmaceutical composition further contains a buffer.

6. The pharmaceutical composition according to Claim 5, wherein the buffer is 1N NaOH.

7. The pharmaceutical composition according to Claim 5, wherein the buffer is present in an amount sufficient to adjust the pH of the pharmaceutical composition to a pH in the range of from pH 3.5 to pH 6.5, or
wherein the buffer is present in an amount sufficient to adjust the pH of the composition to a pH in the range of from pH 4.5 to pH 5.5.

8. The pharmaceutical composition according to Claim 1, wherein the pharmaceutical composition exhibits a shelf-life at 40°C at 75% relative humidity conditions of at least 3 months.

9. The pharmaceutical composition according to Claim 1; wherein the tauroursodeoxycholic acid is in a concentration in the range of from 1 mg/mL to 25 mg/mL, based on the total volume of the composition.

10. The pharmaceutical composition according to Claim 2; wherein the tauroursodeoxycholic acid is in a concentration in the range of from 5 mg/mL to 10 mg/mL.

11. A pharmaceutical dosage form comprising the pharmaceutical composition according to any of claims 1.

12. The dosage form according to claim 11, which is adapted for nasal administration.

13. The pharmaceutical composition according to Claim 1 or the pharmaceutical dosage form according to Claim 11 for use in the treatment of depression.

14. The pharmaceutical composition or the pharmaceutical dosage form for use according to claim 13, wherein the depression is selected from the group consisting of major depressive disorder, unipolar depression, treatment-refractory depression, resistant depression, anxious depression and bipolar depression,

preferably wherein the depression is selected from the group consisting of resistant depression or treatment refractory depression.