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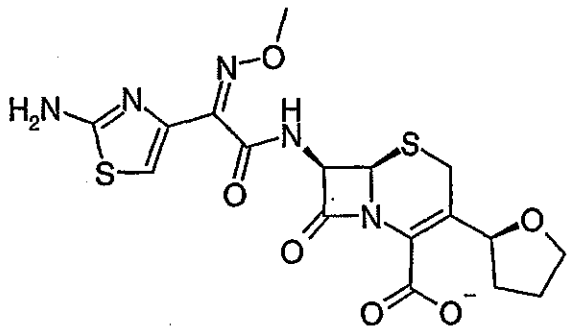
(54) Title: FORMULATIONS COMPRISING A CEPHALOSPORIN COMPOUND AND THEIR USE TREATING BACTERIAL INFECTIONS IN CATS AND DOGS

(57) Abstract: Formulations containing an antibacterial alkali metal salt of a cephalosporin compound and methods of treating or preventing bacterial infections in dogs and cats.

FORMULATIONS COMPRISING A CEPHALOSPORIN COMPOUND AND THEIR USE TREATING BACTERIAL INFECTIONS IN CATS AND DOGS

FIELD OF INVENTION

This invention relates to stable lyophilized formulations containing an antibacterial alkali metal salt of a cephalosporin compound, Compound I, wherein M^+ is a cation, Na^+, K^+ or Li^+ (hereinafter "Compound I"). In particular, the invention relates to stable lyophilized formulations of Compound I, wherein M^+ is Na^+, (6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino) acetyl]amino]-8-oxo-3-[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt. The invention also relates to aqueous formulations of Compound I.



I

M^+

The invention is also directed to methods for treating bacterial infections in dogs and cats by administering a compound of Formula I.

BACKGROUND OF THE INVENTION

Cephalosporins are widely used and therapeutically important antibiotics. The compounds of Formula I are broad spectrum cephalosporin antibacterials and are, therefore, useful in the treatment of bacterial infections in animals. (US 6,020,329, Col. 1, lines 13-14). In particular, Compound I is targeted for dogs and cats with indications for treatment of bacterial infections of the skin, soft tissue, periodontal and urinary tract.

Compound I, wherein M is Na^+, and the preparation thereof, are disclosed in U.S. Patent Nos. 6,001,997, 6,020,329 and 6,077,952. The text of the aforementioned patents and all other references cited in this specification are hereby incorporated by reference in their entirety.

Cephalosporin formulations are generally, however, unstable and a variety of different methods exist to increase stability including, *inter alia*, the adjustment of pH, crystallization, lyophilization, and the addition of stabilizers, such as sugars.

Cephalosporins may be somewhat stabilized within a certain pH range. The optimum pH range varies widely and is unpredictable for different classes of cephalosporins, requiring experimentation and stability tests. For example, Nassar et al., U.S. Pat. No. 5,401,842, disclosed formulations of crystalline cefepime salt buffered with trisodium orthophosphate, sodium bicarbonate, sodium citrate, N-methyl-glucamine and L(+) arginine to a pH of 3.5 to 7.0.

K.A. Conners et al. disclose that cephalothin has a broad stability range from a pH of between 2 to 8. Cepharadine, however, stabilizes at a more acidic pH between 1 to 5. Stability for Cefotaxime is achieved in the pH range of 3 to 7. (K.A. Connors, et al. Chemical Stability of Pharmaceuticals, John Wiley & Sons, New York, 1986, p305).

In some cases, cephalosporin formulations were stabilized by crystallization and lyophilization. For example, Gotschi, U.S. Pat. No. 5,138,066, describes formulations for parenteral administration as lyophilizates or dry powders for dilution with pharmaceutical carriers such as water or isotonic saline.

Bornstein et al, U.S. Pat. No. 4,002,748, disclose methods of preparing essentially amorphous cefazolin by utilizing certain lyophilization techniques, while Daugherty, EP 0327364, describes a lyophilization method to prepare formulations of a crystalline solvate of a 1-carbacephalosporin.

Some cephalosporins may be stabilized by addition of a variety of different sugars. Whether a certain sugar will stabilize a particular cephalosporin, however, is unpredictable. Furthermore, the ratio of sugar to cephalosporin to achieve optimum stability is also unpredictable. For example, Shamblin et al. disclose that the stability of amorphous cefoxitin sodium was improved by a factor of two when co-lyophilized with sucrose. The stability of cefoxitin was not affected, however, when co-lyophilized with trehalose. S.L. Shamblin, B.C. Hancock, M.J.Pikal, The Chemical Stability of Amorphous Cefoxitin Sodium in the Presence of Glassy Stabilizers, AAPS Pharm. Sci. Vol. I, Issue 4, 1999.

Similarly, Shima et al., EP 0134568B1, disclose that sugar (glucose, fructose or maltose) or an alkali metal salt of a mineral acid or carboxylic acid

stabilized a specific lyophilized cephalosporin at a 0.01:1 to 0.5:1 weight ratio of stabilizer:cephalosporin. Mannitol, however, was not effective in stabilizing the disclosed cephalosporin compound.

Likewise, Almarsson et al., Tetrahedron 56 (2000) 6877-6885, disclose that sucrose improved chemical stability of a *beta*-lactam compound at a sucrose/drug ratio of 0.1:1 to 0.5:1.

Yoshioka, Y. et al., Pharm, Res. 17 (2000), 925-929, disclose the stability of cephalothin in the presence of dextran at a dextran/cephalothin ratio of 200:1.

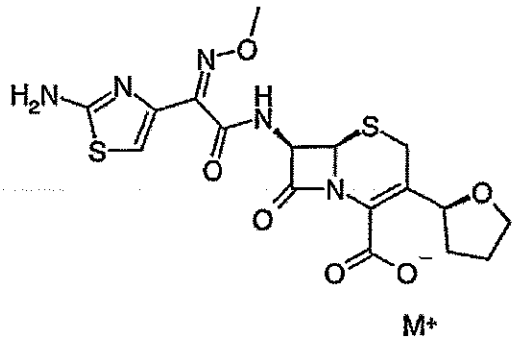
Conversely, Hirai et al., U.S. Patent No. 4,418,058, disclose that an excess amount, greater than 1:1, of a variety of different sugars or sugar alcohols adversely affected chemical stability of cephalosporins. Good stabilizing results were obtained, however, when the amount of added sugar or sugar alcohol was 0.1 to 1 sugar/cephalosporin.

Consequently, one of ordinary skill in the art cannot, in general, predict whether the addition of a particular sugar to any particular cephalosporin will achieve stability. Moreover, the optimum ratio of sugar:cephalosporin is also highly variable and unpredictable, absent experimentation. Furthermore, as discussed above, the optimum pH range of stability for a particular cephalosporin is also unpredictable.

A method of administration for Compound I is by parenteral administration. Other modes of administration include oral and topical. (US 6,020,329, Col. 15, lines 1-2 Compound I is unstable, as both a solid and an aqueous solution. Furthermore, Compound I is hygroscopic. Consequently, a formulation and method for stabilizing Compound I would be a useful addition to the art.

SUMMARY OF THE INVENTION

In a first aspect of the invention, there is provided a lyophilized pharmaceutical composition comprising a compound of Formula I,



M+

I

wherein M^+ is Na^+, K^+ or Li^+, and a preservative selected from methyl paraben, propyl paraben and a mixture of methyl and propyl parabens, wherein the composition has a solution pH in the range of 6.0 to 7.5.

In a preferred embodiment, M^+ is Na^+.

In a preferred embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable buffer.

In a preferred embodiment, the compound of Formula I is amorphous (6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino) acetyl]amino]-8-oxo-3-[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt.

In a preferred embodiment, the pharmaceutical composition further comprises a pharmaceutical acceptable buffer.

In a more preferred embodiment, the buffer is carbonate, phosphate, citrate or acetate, and the pH within a range of 6.0 to 7.5.

In a preferred embodiment, the buffer is citrate.

In a preferred embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable bulking agent.

In a more preferred embodiment, the bulking agent is selected from sugars, polyalcohols, amino acids, polymers, polysaccharides or inorganic salts.

In a preferred embodiment, the composition has a bulking agent/compound of Formula I ratio greater than 1.0, but less than 100.

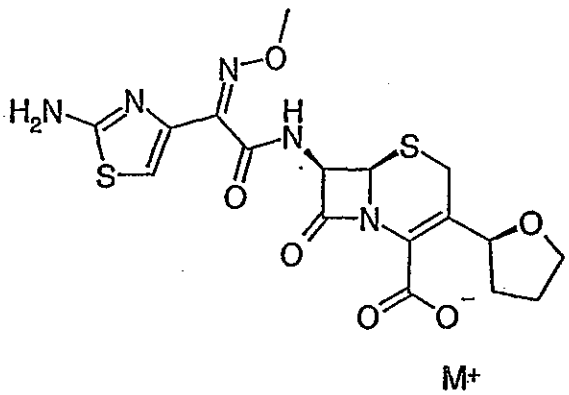
In a more preferred embodiment, the ratio is greater than 1, but less than 10.

In a morr preferred embodiment, the bulking agent is sucrose and the composition has a sucrose/compound of Formula I ratio of 3.

In a preferred embodiment, a pharmaceutical further comprises a pharmaceutically acceptable preservative.

In a more preferred embodiment, the preservative is a combination of methyl and propyl parabens.

In a second aspect of the invention, there is provided a kit comprising a therapeutically effective amount of a lyophilized pharmaceutical composition comprising a compound of Formula I,



I

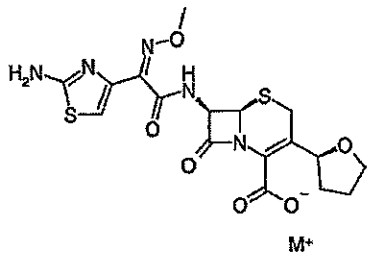
M+

wherein M^+ is Na^+, K^+ or Li^+ and a preservative selected from methyl paraben, propyl paraben and a mixture of methyl and propyl parabens, wherein the composition has a solution pH in the range of 6.0 to 7.5, an aqueous pharmaceutically acceptable diluent, and a first and second container means for containing the composition (a) and the diluent (b), wherein the first container is adapted to receive the diluent from the second container.

In a preferred embodiment, M^+ is Na^+.

In a more preferred embodiment, the kit comprises a pharmaceutically acceptable buffer.

In a third aspect of the invention, there is provided a use of a compound of Formula (I)



I

wherein M^+ is Na^+, Li^+ or K^+ in the manufacture of a medicament for treating or preventing a condition caused by a bacterial infection in dogs and cats.

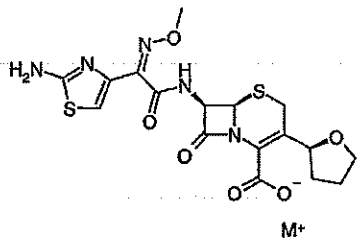
In a preferred embodiment, M^+ is Na^+.

In a more preferred embodiment, the compound of Formula (I) is amorphous (6*R*,7*R*)-7-[[2*Z*)-(2-amino-4-thiazolyl)(methoxyimino) acetyl[amino]-8-oxo-3-[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt.

In a preferred embodiment, the condition is a skin, soft tissue or urinary trout bacterial infection.

In a more preferred embodiment, the condition or infection is caused by or complicated by Gram positive or Gram negative bacteria.

In a fourth aspect of the invention, there is provided a use of a lyophilized pharmaceutical composition comprising a compound of Formula I according to the first aspect.



I

In one embodiment, the condition is a skin, soft tissue or urinary tract bacterial infection.

In another embodiment, the condition or infection is caused by or complicated by Gram positive or Gram negative bacteria.

The term "about," as used herein, is defined as a pH of 0.5 above or below the designated upper and lower pH units.

The term "aqueous pharmaceutically acceptable diluent" means water or other pharmaceutically acceptable aqueous solutions containing one or more pharmaceutically acceptable excipients for use in making the compositions of the invention (e.g. isotonic solution of sodium chloride, water for injection with ethanol or phosphate, acetate or citrate buffer, and water for injection with benzyl alcohol).

The term "Na^+," as used herein, is defined as a sodium cation.

The term "K^+," as used herein, is defined as a potassium cation.

The term "Li^+," as used herein, is defined as a lithium cation.

The term "composition", as used herein, encompasses, *inter alia,* (1) solutions comprising Compound I or (2) dry lyophilized residues of such solutions. The solutions may contain one or more optional agents which aid in stabilizing dissolved Compound I and/or that facilitate re-dissolution, upon reconstitution of the lyophile created after lyophilizing solution (1). Such optional agents include, *inter alia,* bulking agents, preservatives, and buffers, as further disclosed herein.

The term "Compound I" is limited to the pharmaceutically acceptable alkali metal salts of Compound I, wherein M^+ is Na^+, K^+ or Li^+, and in particular, includes Compound I, (6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino) acetyl]amino]-8-oxo-3-[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt), wherein M^+ is Na^+.

The term "lyophilization" means the process of freeze-drying a composition as known in the art. "Lyophilized" and "freeze-dried" are used herein as synonyms.

The terms "pharmaceutical" and "pharmaceutically" and the like are meant to refer to applications in both human and veterinary fields.

DETAILED DESCRIPTION OF THE INVENTION

Compound I is a broad spectrum cephalosporin antibacterial targeted for mammals, in particular, dogs and cats. The preparation of Compound I wherein M^+ is Na^+ (hereinafter the "sodium salt") is described in U.S. Patent Nos. 6,001,997,

6,020,329, 6077,952, as well as EP1178049A1; incorporated herein by reference in their entirety. The K^+ and Li^+ salts of Compound I may be prepared by one of ordinary skill in the art, as described in the preparation of the sodium salt of compound I, but substituting an appropriate K^+ or Li^+ salt.

The antibiotic compounds of the present invention are active against a wide range of organisms, including both Gram-negative organisms (*e.g. E.coli*), and Gram-positive organisms, (*e.g.S. aureus*). (US 6,020,329, Col 17, lines 28-31). Compound I can be used to treat, *inter alia,* bacterial infections of the skin, soft tissue and urinary tract. For example, conditions or infections caused by or complicated by Gram positive and/or Gram negative bacteria are: canine pneumonia, feline pneumonia, canine pyoderma, feline pyoderma, pasteurellosis, pneumonia, otitis media, sinusitus, bronchitis, tonsillitis, and mastoiditis associated with infection by *Staphylococcus spp.* (*Staphylococcus intermedius, Staphyloccus aureus*), *Escherichia coli, Streptococcus spp.* (*Beta Hemolytic Streptococcus spp.*), *Pasteurella multocida, Bacteriodes spp., Fusobacterium spp., Porphyromonas spp., Prevotella spp., Peptostreptococcus spp.*, and *Clostridium spp.*, uncomplicated skin and soft tissue infections, abscesses, osteomyelitis, and puerperal fever associated with infection by *Staphylococcus aureus, S. intermedius, coagulase-positive staphylococci, S. epidermidis, S. hemolyticus, Streptococcus ssp, Streptococcal groups C-F (minute-colony streptococci), viridans streptococci,* uncomplicated acute urinary tract infections associated with infection by *Staphylococcus ssp or E. coli.;* odontogenic infection associated with infection by *viridans streptococci;* urinary tract infection in dogs and cats associated with infection by *E. coli;* skin and soft tissue infections in dogs and cats associated with infection by *Staph. epidermidis, Staph. intermedius, coagulase neg. Staph. or P. multocida*; infections of the oral cavity in dogs and cats associated with infection by *Alcaligenes spp., Bacteroides spp., Clostridium spp., Enterobacter spp., Eubacterium, Peptostreptococcus, Porphyromonas, or Prevotella.*

It was determined that the compounds of Formula I, as well as similar compounds disclosed in U.S. Patent Nos. 6,001,997, 6,020,329 and 6,077,952, exhibit an unexpectedly long half-life in dogs and cats, especially in view of comparable antibiotics. For example, Table I lists well-known antibiotics and their respective half-lives in different mammals, such as in mice, rats, dogs and cats.

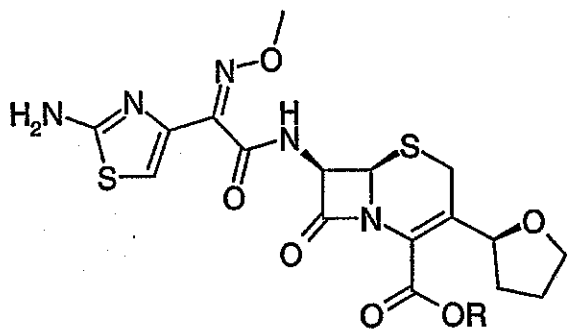
TABLE I: Half-life of Known Antibiotics

Compound	Mouse $t_{1/2}$ (h)	Rat $t_{1/2}$ (h)	Canine $t_{1/2}$ (h)
Cefpodoxime	0.68 (PO)	1.4 (PO)	2.4 (PO)
Ampicillin		0.84 (IM)	0.64 (IM)
Cefamandole		0.5 (IM)	0.82 (IV)
Cefazolin		0.66 (IM)	1.11 (IV)
Cefuroxime	0.32 (SC)	0.4 (IM)	0.93 (IM)
Cephalordine		0.5 (IM)	0.97 (IM)
Cephalothin	0.208 (IM)	0.4 (IM)	1.06 (IM)
Cephadrine		0.82 (PO)	3.64 (PO)
Erythromycin	0.65 (IV)	1.27 (IV)	1.72 (IV)
Oleandomycin	0.7 (IV)	0.93 (IV)	1.53 (IV)
Tylosin		0.4 (IV)	1.24 (IV)

(Cefpodoxime data from "Abstracts of the 1996 ICAAC"; Abstract 593. All other data compiled from: "CRC Handbook of Comparative Pharmacokinetics and Residues of Veterinary Antimicrobials", J. Edmond Riviere; Arthur L. Craigmill, Stephen F. Sundlof CRC Press 1991; Routes of administration: "IV" - intravenous; "IM" = intramuscular; "PO" = per os; "SC" = subcutaneous)

A number of cephalosporin derivatives, including compounds of Formula I, were disclosed in International Patent Application publication number WO 92/01696 and by Bateson *et al* in The Journal of Antibiotics, Feb.1994, vol.47, no.2, at pages 253-256. Various mouse data are also disclosed in the latter paper. Both of these publications are herein incorporated in their entirety.

In particular, following administration of the compound of Formula II, the half-life in the mouse and rat were determined to be 2.2 and 3.9 hours, after *per os* administration, respectively. Unexpectedly, however, in dogs and cats the half-life was in both cases dramatically increased, as is demonstrated below in Table II.



II

TABLE 2: Half-life in Dogs and Cats

Experiment	Species	Route	Dose[1] (mg/kg)	Compound	Half-life
1	Dog	IV	1	Compound of Formula I, M^+ = Na	6.9 days
2	Dog	SC	8	Compound of Formula I, M^+ = Na	4.7 days
3	Dog	PO	1	Compound II , R = pivaloyloxymethyl	6.0 days
4	Cat	SC	8	Compound of Formula I, M^+ = Na	6.3 days

Note [1] Dose expressed as corresponding free acid : *i.e.* M^+ = H. Concentrations measured w.r.t. free acid.

EXPERIMENTAL DETAILS

a. Pharmacokinetics

Experiment 1: Intravenous Dog

A male dog was dosed intravenously with an aqueous solution of Compound I. Blood plasma was sampled at times up to 28 days post dosing. Plasma samples were extracted and assayed to determine the concentration by both bioassay and HPLC as follows:

1mL of plasma (or standards of spiked dog plasma) were acidified to a pH of less than 3 with hydrochloric acid, then shaken with 26 mL of ethyl acetate. The layers were separated by centrifugation. 22 mL of the organic layer was transferred into a fresh container and 2.0 mL of 0.1 M phosphate buffer, pH 7.0, was added. After shaking and centrifugation, the aqueous phase was recovered and assayed. Following processing, samples (and standards) were assayed by hole-in-the-plate microbiological bioassay on large plates (200 mL Mueller Hinton agar) seeded with *M. luteus.* Samples were also assayed by HPLC (μBondapk –C18 column eluted with acetonitrile- 0.05M sodium acetate pH 5.0, 15:85, with UV detection at 256nm). Good agreement was obtained between the two assay methods, and the half-life was calculated from bioassay results using standard pharmacokinetic methods.

Experiment 2: Subcutaneous Dog

Two dogs were dosed with compound of Formula I by subcutaneous injection. Blood plasma was sampled at times up to 28 days post-dosing. Plasma samples and appropriate standards were prepared by deproteination by the addition of an equal volume of acetonitrile and centrifugation (3000 r.p.m. for 10 min.). Supernatant

was assayed by a specific HPLC method to determine the concentration (μBondapk –C18 column eluted with acetonitrile- 0.05M sodium acetate pH 5.0, 15:85, at 1.0 mL/min with UV detection at 256nm). Pharmacokinetic parameters were calculated using the program PCNONLIN.

Experiment 3: *per os* Dog

Six dogs were dosed orally with the pivaloyloxymethyl-ester pro-drug, compound of Formula II, and the resulting plasma concentrations were determined by both bioassay and HPLC. Following dosing, blood plasma was sampled up to 696 hours (29 days). Plasma samples and appropriate standards (1mL) were first acidified to a pH less than 3.0 with hydrochloric acid then shaken with 30 mL ethyl acetate. The layers were separated by centrifugation then 25mL of the organic layer was removed. 2mL of 0.1M phosphate buffer pH 7.0 was added to the ethyl acetate and shaken to effect a back extraction. After separation of the layers, the aqueous phase was removed and used for the assays. Following processing, samples (and standards) were assayed by hole-in-the-plate microbiological bioassay on large plates (200 mL Mueller Hinton agar) seeded with *M. luteus.* Samples were also assayed by HPLC (μBondapk –C18 column eluted with acetonitrile- 0.05M sodium acetate pH 5.0, 15:85,at 1.5 mL/min with UV detection at 256nm). There was good agreement between the two assay methods (r=0.9716) half-life was calculated from the bioassay data.

Experiment 4: Subcutaneous Cat

Four cats were dosed at 8 mg/kg by subcutaneous injection of Compound I. Blood samples were taken at intervals to 35 days post-dosing and the plasma assayed to determine the concentration of the corresponding free acid by HPLC/MS/MS. Plasma samples (100 mL) were aliquotted into centrifuge tubes, then 400 mL of acetonitrile was added. Following vortexing (60 sec.) and centrifugation (20,800 x g for 10 minutes), 0.450 mL of the supernatant was transferred into clean centrifuge tubes, and evaporated to dryness at approximately 50°C under N_2. Dried samples were reconstituted in 0.100 mL of mobile phase (15/85 v/v acetonitrile/10 mM HCO_2NH_4, pH 3.0), vortexed for 1 minute, centrifuged at 3,000 rpm for 2 minutes, and transferred to an autosampler vial. Single replicates of plasma were analyzed by LC-MS/MS for concentration of compound. Sample analysis was performed on a SCIEX API 365 or 3000 HPLC/MS/MS system. The column effluent was connected to a Turbo-ionspray source set at 4500 V. The collision gas was set

to a value of 3. Positive ions were generated in the source and sampled through an orifice into the quadrupole mass filter. The mass spectrometer was adjusted to monitor the precursor and product ions as follows: m/z 454.0 -> m/z 241.0. Half-life was calculated using pharmacokinetic program WINNONLIN v2.1 and determined to be 8.39 +/- 0.97 days.

b. Efficacy

In an experimentally induced skin infection model study, five out of six dogs had complete clearance of *Staphylococcus intermedius* 15 days after a single administration of 8 mg/kg of the Compound I.

In a separate study, following a single administration of 8 mg/kg Compound I to healthy dogs, there was a significant reduction of the populations of pathogenic *Staphylococci* for four weeks compared to non-treated control animals.

In an experimentally induced abscess model study in cats, there was a substantial reduction in the numbers of *Pasteurella multocida, Clostridium perfringens*, and *Bacteriodes fragilis* bacteria 14 days after a single administration of 8 mg/kg Compound I

The above half-life results, together with the potency of the compounds of Formula I, demonstrates that one administration of an equivalent of ca. 4-12 mg/kg of Compound I, (*e.g.*Na salt of compound of Formula I), given by injection (*e.g.* intramuscularly, subcutaneously or intravenously), to a cat or dog would advantageously provide an efficacious concentration for 7-21 days. This represents a novel and very convenient treatment regime for veterinary practitioners and cat and dog owners alike.

It has been determined, however, that Compound I is unstable both as a solid and a liquid. In evaluating possible formulations, stability experiments were conducted. As used herein, "stable" or "stabilized" means less than or equal to about 10% decomposition of Compound I.

While many cephalosporins may be stabilized by crystallization, it has been determined that Compound I is not particularly amenable to crystallization techniques on a commercial scale. Consequently, Compound I is in an amorphous state and is hygroscopic. It was determined that optimal long-term stability of Compound I is achieved at a low residual water content. Accordingly, lyophilization of formulations of Compound I provide preferred stability.

With respect to the present invention, stable formulations of Compound I were developed, overcoming the inherent stability problems previously hampering long-term storage goals. It was determined that Compound I could be stabilized and formulated into injectable preparations by formulating Compound I with an aqueous pharmaceutically acceptable diluent, such that the pH is in the range of about 5.0 to about 8.0.

For example, formulations are prepared by dissolving a therapeutically effective amount of the sodium salt of Compound I in an aqueous pharmaceutically acceptable diluent and adjusting the pH, if necessary, to within the range of about 5.0 to about 8.0. Alternatively, the free acid form of Compound I *(i.e.* the carboxylate, instead of the salt) may be utilized as starting material. A suspension or solution of the free acid may be titrated with, for example, sodium hydroxide, forming the sodium salt of Compound I. Adjustments to pH may be conducted as described above.

Aliquots of the resulting solution, the quantity of which is dependent upon the ultimate desired reconstituted concentration of Compound I, are clarified and sterile filtered and aseptically transferred to containers appropriate for lyophilization, (*e.g.* vials), and partially stoppered with lyo-stoppers. As described hereinafter, the formulation is cooled to freezing, subjected to lyophilization in a manner conventional per se in the art and hermetically capped, forming a stable, dry lyophile formulation. In a preferred embodiment, the composition has a low residual water content, less than 1% by weight, based on the weight of the lyophile. In a more preferred embodiment, the composition has a residual water content level of less than 0.5% by weight.

As used herein, a "therapeutically effective amount" for a dosage unit may typically about 50 to about 500 mg of active ingredient. (US 6020,329; Col 16, line 3). The dose may vary, however, depending on the species, variety, etc. of animal to be treated, the severity and type of infection and the body weight of the animal. Accordingly, based upon body weight, typical dose ranges of the active ingredient may be from about 0.01 to about 100 mg per kg of body weight of the animal. Preferably, the range is from about 1 to about 20 mg per kg of body weight, and more preferably, from about 4 to about 12 mg per kg of body weight. (PCS10965; p7, lines7-11)

The veterinary practitioner, or one skilled in the art, will be able to determine the dosage suitable for the particular individual patient, which may vary with the species, age, weight and response of the particular patient, as well as the bacterial species involved. The above dosages are exemplary of the average case. Accordingly, higher or lower dosage ranges may be warranted, depending upon the above factors, and are within the scope of this invention.

Compounds of Formula I may be administered either alone or in combination with one or more agents used in the treatment or prophylaxis of disease or in the reduction or suppression of symptoms. Examples of such agents (which are provided by way of illustration and should not be construed as limiting) include antiparasitics, eg arylpyrazoles such as fipronil, lufenuron, imidacloprid, avermectins (eg abamectin, ivermectin, doramectin, selamectin), milbemycins, organophosphates, pyrethroids; antihistamines, eg chlorpheniramine, trimeprazine, diphenhydramine, doxylamine; antifungals, eg fluconazole, ketoconazole, itraconazole, griseofulvin, amphotericin B; antibacterials, eg enroflaxacin, marbofloxacin, ampicillin, amoxycillin; anti-inflammatories eg prednisolone, betamethasone, dexamethasone, carprofen, ketoprofen; steroids or other antipruritic agents; dietary supplements, eg gamma-linoleic acid; and emollients. Therefore, the invention further provides for uses, etc., of compounds of formula (I) and one or more selected compounds from the above list as a combined preparation for simultaneous, separate or sequential use in the treatment of diseases or conditions according to the invention.

The composition of the formulations may optionally contain auxiliary ingredients, as known in the art, such as buffers, bulking agents, diluents, co-solvents, solvents, preservatives, chelating agents, antioxidants, tonicity adjusters, whose presence may help to provide a rapidly soluble freeze-dried product or extend the storage time of the formulation.

An example of a possible solvent and co-solvent is ethanol. An example of a chelating agent is ethylenediaminetetraacetic acid. An example of an antioxidant is ascorbic acid. An example of a tonicity adjuster is dextrose. Furthermore, the compound of Formula I may be the sole therapeutic agent in the compositions of the invention or a combination with other antibiotics or with a β-lactamase inhibitor may be employed. (US 6,020,329; Col 16, lines 15-18)

Unlike most cephalosporins with typical broader pH ranges of stability, it was determined that formulations of Compound I with or without various buffers have a

relatively narrow range of stability between a pH of about 5.0 to about 8.0. In particular, and in a preferred embodiment, optimal solution and solid state stability are achieved at a pH of about 6.0 to about 7.5. Adjustment of pH may be accomplished by either titrating to the desired pH range with, for example, a 10% solution of sodium hydroxide or hydrochloric acid, or using an appropriate buffer. Typical buffers include phosphate, acetate, citrate, carbonate, and glycine. In a preferred embodiment, phosphate is used as a buffer. In a more preferred embodiment, citrate is used as a buffer.

The water-soluble bulking agent suitable for use in the present invention can be any of the pharmaceutically acceptable inert solid materials typically used for lyophilization. Bulking agents may improve stability and/or provide a more rapidly soluble freeze-dried product. Such bulking agents include, for example, sugars such as glucose, maltose, sucrose and lactose; polyalcohols such as sorbitol and mannitol; amino acids such as glycine; polymers such as polyvinylpyrrolidone; polysaccharides such as dextran; certain inorganic salts such as sodium or potassium phosphates, or sodium chloride.

The ratio of the weight of the bulking agent to the weight of Compound I used in the compositions of the present invention should generally be within the range of from about 0.01 to about 100, depending upon the bulking agent utilized. In a preferred embodiment, polyhydroxy compounds are the bulking agent of choice. In a more preferred embodiment, sucrose is the bulking agent and was found to stabilize the sodium salt of Compound I when co-lyophilized therewith.

The optimum ratio of sucrose/Compound I, however, was unpredictable, absent experimentation. For example, it was determined that, compared to formulations without sucrose additions, a relatively small amount of sucrose (*e.g.* sucrose/sodium salt of Compound I ratio of 0.4) increased the extent of degradation of the sodium salt of Compound I. On the other hand, a sucrose/sodium salt of Compound I ratio of 1 exhibited similar stability as formulations wherein no sucrose was added. Ratios greater than 1.0, however, increased the stability of formulations of the sodium salt of Compound I. In a preferred embodiment, the sucrose/sodium salt of Compound I ratio ranges from greater than 1.0 to about 10. In an even more preferred embodiment, the sucrose/sodium salt of Compound I ratio is about 3.

Higher sucrose/Compound ratios may be utilized. A high sucrose concentration is limited, however, by practical considerations of viscosity of high concentrated solution, impacting syringibility of the reconstituted solution. Furthermore, high sucrose concentrations may create injection site intolerance for injectable preparations. As a general rule, viscosity of 25-30 mPa•s (millipascals • second), wherein "•" is defined as "multiplied by", can be considered as an upper limit for injectable preparations in the pharmaceutical industry. This translates to an approximate maximum 60% sucrose solution at 40°C. (M.Mathlouthi, J. Génotelle, in: M.Mathlouthi, Sucrose. Properties and Applications, Blackie Academic & Professional, London, 1995, p.137). For example, if the concentration of Compound I in solution is 6% by weight, the acceptable sucrose/Compound I ratio would be 10:1. If Compound I concentration is 3%, the acceptable sucrose/Compound I ratio would be 20:1. These are but two examples of many possible ratios.

Antimicrobial preservatives are frequently added to pharmaceutical formulations to prevent microbial contamination. As used herein, the word "preservative" means a compound, or combination of compounds, added to prevent or inhibit the growth of micro-organisms which could present a risk of infection or degradation of the medicinal product. Generally, the level of efficacy obtained varies according to the chemical structure of the preservative, its concentration and the physical and chemical characteristics of the medicinal product (especially pH). The design of the pack and the temperature at which the product is stored will also affect the level of activity of any antimicrobial preservatives present. Useful preservatives may include *m*-benzoic acid and its salts, sorbic acid and its salts, alkyl esters of parahydroxybenzoic acid, phenol, chlorobutanol, benzyl alcohol, thimerosal, benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, *m*-cresol and chlorocresol. Mixtures of the aforementioned preservatives may also be employed.

In the present invention, formulations of Compound I containing antimicrobial preservatives were effective in satisfying United States Pharmacopeia (hereinafter "USP") criteria for antimicrobial effectiveness. In particular, various formulations of the following preservatives were found to satisfy USP criteria, for example, methyl paraben, propylparaben, *m*-cresol and benyzl alcohol. To satisfy European Pharmacopeia (hereinafter "EP") criteria for antimicrobial effectiveness,

however, other preservatives were more appropriate (*e.g.* benzethonium chloride and combinations of several preservatives such as methylparaben, propylparaben and benzyl alcohol, in one combination, and methyl paraben and *m*-cresol in another combination.

Formulations of Compound I can be isolated by drying, preferably by lyophilization as known in the art. Usually the lyophile formulations are produced with ampule lyophilization, vial lyophilization, tray lyophilization, or like conventional methods by cooling the formulations at subzero temperature to freezing. The frozen material is then dried under vacuum by subliming the water component originally contained in the solution as a solvent, thus leaving a solid lyophilized cake. Thus, for example, the excipients described above, Compound I, or the pharmaceutically acceptable salt of Compound I, are successively dissolved under stirring in a suitable amount of water for injections. Then, further water is added to reach the desired final volume. The resulting solution is clarified, sterile filtered and aseptically distributed in sterile containers (*e.g.* vials) of desired capacity. Freeze-drying the solution is then performed and the vials are hermetically sealed according to conventional procedures.

The lyophilized drug product is amorphous Compound I, and more preferably the sodium salt thereof. When a product solution is required, it can be reconstituted by dissolving the dry formulation in water for injection, bacteriostatic water for injection or another pharmaceutically acceptable diluent (*e.g.* isotonic solution of sodium chloride, water for injection with ethanol or citrate buffer, and bacteriostatic water for injection with benzyl alcohol) in an amount sufficient to generate a solution of the required strength for parenteral administration to patients.

An amount of Compound I may be administered such that the composition provides the desired therapeutical effect as disclosed in U.S. Patent Nos. 6,001,997, 6,020,329 and 6,077,952. The injectable reconstituted solutions of the invention may be administered, according to a variety of possible dose schedules.

EXAMPLES

To confirm the advantageous effects of the present invention, the sodium salt of Compound I, was formulated into lyophilized injectable preparations and stability of the formulations was measured.

The examples below are intended to illustrate particular embodiments of the invention and are not intended to limit the specification, including the claims, in any manner.

A. Stabilization of lyophilized formulations by pH adjustment.

The formulations described in Tables 3-6, demonstrate the increased instability of compositions, both buffered and unbuffered, outside the pH range of about 5.0 to about 8.0. As set forth below in Tables 3 and 4, the sodium salt of Compound I was dissolved in either deionized water or in citrate buffer solutions at 50 mg/mL.

For formulations 1-5, solution pH was adjusted with a 10% solution of hydrochloric acid after dissolution of the sodium salt of Compound I (TABLE 3). For formulations 6-13, a buffered solution was prepared with citrate and adjusted with a 10% sodium hydroxide solution, upon which the sodium salt of Compound I was dissolved therein (TABLE 4). One mL aliquots of solutions of the sodium salt of Compound I were filled in 10 mL vials, and lyophilized using a FTS Kinetics freeze dryer (FTS Systems, Stone Ridge, New York). During lyophilization, the compositions were frozen using a two-step freezing protocol (at -25ºC and -40ºC), followed by primary drying at -27ºC for approximately 22 hours, followed by a secondary drying with increased steps of temperature to 0ºC, 25ºC and 50ºC. The pressure during primary and secondary drying was set at 60 millitorr.

Examples of the solution preparation procedures are given below.

TABLE 3. Composition of formulations with HCl.

Formulation #	Compound I (mg/vial)	HCl*	pH
1	50	+	3.5
2	50	+	3.9
3	50	+	4.1
4	50	+	5.1
5	50	-	6.0

"+" sign means that HCl was added to the formulation whereas "-" sign indicates that HCl was not added.

TABLE 4. Composition of formulations with Citric Acid.

Formulation #	Compound I (mg)	Citric acid (mg)	pH*
6	50	9.6	5.0
7	50	9.6	5.5
8	50	9.6	5.7
9	50	9.6	6.0
10	50	9.6	6.2
11	50	9.6	6.5
12	50	9.6	6.8
13	50	9.6	7.0
14	50	-	6.1

*pH of the solutions were adjusted to the specified values with 10% sodium hydroxide solution prior to lyophilization.

The samples were stored at 40ºC for 12 weeks. The remaining amount of Compound I was then measured by reverse-phase High Pressure Liquid Chromatography ("HPLC"), using a Waters (Milford, MA) HPLC system with an Ultraviolet ("UV") detector set at 256 nm and a Kromasil C4 column (MetaChem Technologies Inc., Torrance, CA). A gradient method was utilized with mobile phase A consisting of 9:1 ratio v/v 0.025M sodium phosphate buffer solution, pH 6.5: acetonitrile, and mobile phase B consisting of 4:6 ratio v/v 0.025M sodium phosphate buffer solution, pH 6.5: acetonitrile.

Degradation results are reported at Tables 5 and 6 as percentage (%) of initial purity of Compound I. Degradation of the formulation after 18 months storage at a controlled room temperature of 25ºC *(i.e.* typical shelf storage conditions) was calculated using the pseudo-zero order model and the Arrhenius equation with an activation energy of 10 kcal/mol (*e.g.*, K.A. Connors, Chemical Kinetics, 1990, VCH Publishers, Inc., New York). At this activation energy, the degradation rate constant at 40ºC (k_{40}) is equal to 2.27*k_{25} (k_{25} is the degradation rate constant at 25ºC). A person of ordinary skill in the art would appreciate the reasonableness of this assumption, based on data reported by Pikal *et al.* for other amorphous cephalosporin compounds wherein, k_{40}=2.2*k_{25}. (M.J.Pikal, K.M. Dellerman, Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25ºC: cephalosporins in the solid and aquesous solution states, Int. J. Pharm. 50 (1989) 233-252).

As set forth in Table 5, formulations 4 and 5 (respectively, pH of 5.1 and 6.0, from Table 3) had an acceptable long-term stability, (i.e. degradation after 18 months at 25ºC is less than 10%). Formulations with a pH less than or equal to 4.1, however, demonstrated degradation at greater than 10%, which is typically unacceptable in the pharmaceutical industry for pharmaceutical products. As demonstrated in Table 4, the optimal stability for formulations utilizing a citrate buffer, however, was between pH of about 6.0 to about 7.0.

TABLE 5. Percent Compound I after 12 weeks storage at 40°C and estimated shelf life at controlled room temperature 25ºC

Formulation #	12 weeks (40ºC)	% Degradation after 12 weeks (40ºC)	% Degradation* after 18 months (25ºC)
1	91.3	8.7	19
2	94.3	5.7	15
3	95.9	4.1	11
4	96.9	3.1	8
5	97.2	2.8	7

* Calculated as described in the text

TABLE 6. Percent Compound I after 12 weeks storage at 40°C and estimated shelf life at controlled room temperature 25ºC

Formulation #	12 weeks (40ºC)	% Degradation after 12 weeks (40ºC)	% Degradation* after 18 months (25ºC)
6	97.5	2.5	7
7	98.0	2.0	5
8	98.6	1.4	4
9	98.7	1.3	3
10	98.9	1.1	3
11	99.1	0.9	2
12	99.2	0.8	2
13	99.1	0.9	2
14	99.2	0.8	2

* Calculated as described in the text

Examples of solution preparation for the above stability studies of lyophilized formulations are with specific concentrations as follows:

Example 1

Formulation without pH Adjustment

1.0453 grams of the sodium salt of Compound I were dissolved in 20.0 mL de-ionized water. One mL aliquots of the resulting solution were transferred to 10-mL vials, partially stoppered with lyo-stoppers, lyophilized and hermetically capped, as described hereinabove.

Example 2

Formulation with pH Adjusted by HCl

0.5085 grams of the sodium salt of Compound I were dissolved in 10.0 mL de-ionized water. The solution was titrated with 0.1 N hydrochloric acid to pH 3.87. One mL aliquots of the resulting solution were transferred to 10-mL vials, partially stoppered with lyo-stoppers, lyophilized and hermetically capped, as described hereinabove.

Example 3

Formulation with pH Adjusted by Citrate Buffer

0.6 grams of the sodium salt of Compound I were dissolved in 12 mL of 0.05M citrate buffer at pH of 6.0. The resulting solution was filtered and 1 mL aliquots were transferred to 10-mL vials, partially stoppered with lyo-stoppers, lyophilized and hermetically capped, as described hereinabove.

B. Stabilization of lyophilized formulation of the sodium salt of Compound I by Bulking Agents.

In each experiment, formulations of the sodium salt of Compound I and sucrose, at different sucrose/sodium salt of Compound I ratios (TABLE 7), were lyophilized according to standard industry procedures, as described hereinabove. Formulations 15 to 18 and 19 to 22 were prepared independently for quality control reproducibility purposes. Stability measurements of formulations 19 to 22 were only taken at 12 weeks.

TABLE 7. Composition of formulations

Formulation #	Compound I (mg/vial)	Sucrose (mg/vial)	Sucrose/Compound I Ratio
15	50	0	0
16	50	20	0.4
17	50	50	1.0
18	50	150	3.0
19	50	0	0
20	50	20	0.4
21	50	50	1.0
22	50	150	3.0

The samples were stored at 40ºC for up to 12 weeks. The remaining amount of Compound I was measured by reverse-phase HPLC using gradient solvent method, as described above, and reported as percentage (%) of remaining Compound I. The results, as set forth in TABLE 8, demonstrate that additions of sucrose at a ratio of 3:1 (sucrose/sodium salt of Compound I) improved stability. A lower sucrose/sodium salt of Compound I ratio of 2:5 sucrose was less desirable. Stability of formulation with 1:1 ratio was similar to the formulation without sucrose.

TABLE 8. Percent Compound I after 12 weeks at 40°C

Formulation #	1 week	2 weeks	4 weeks	6 weeks	12 weeks
15	100.0	99.8	99.1	99.0	97.4
16	98.0	97.7	97.4	96.5	94.6
17	99.5	99.3	98.7	98.3	96.9
18	101	101	101	100	99.5
19	-	-	-	-	98.5
20	-	-	-	-	96.5
21	-	-	-	-	97.5
22	-	-	-	-	99.1

An example of solution preparation for the above stability studies of lyophilized formulations are is follows:

Example 4

Formulation with Sucrose

0.4818 grams of the sodium salt of Compound I and 0.1964 grams of sucrose were dissolved in 10 mL of de-ionized water. The resulted solution was filled in 1-mL aliquots in 10-mL vials, partially stoppered with lyo-stoppers and lyophilized, as

described hereinabove. At the end of the lyophilization cycle, the vials were hermetically capped.

C. Preservatives.

Generally, preservatives are not required for cephalosporin formulations, including formulations of Compound I. Formulations stored in multi-dose containers versus single dose containers, however, require the addition of preservatives to satisfy antimicrobial effectiveness in vitro. Various formulations prepared in accordance with the working Examples hereinafter were tested for antimicrobial activity in vitro. Antimicrobial Effectiveness Tests (hereinafter "AET") were performed according to European Pharmacopoeia (EP) Procedures (European Pharmacopoeia, 3d edition, Supplement 201, Council of Europe, Strasbourg) and United States Pharmacopeia (USP) procedures (U.S. Pharmacopeia, and National Formulary USP24 NF19, 2000, United States Pharmacopeia Convention Inc., Rockville, MD). The effectiveness of formulations containing preservatives and the sodium salt of Compound I is demonstrated in the following examples:

Example 5

AET of the sodium salt of Compound I and methylparaben (1.8 mg/mL)

AET were conducted on a solution containing the sodium salt of Compound I (80 mg/mL) and methylparaben (1.8 mg/mL) in 20 mM citrate buffer. The results of AET are provided in Table 9. The formulation satisfied USP criteria (see Table 14 for acceptable criteria) for all microorganisms. Furthermore, the formulation satisfied criteria A for all microorganisms, except for *S. aureus* at 6 and 24 hours time points.

TABLE 9. Log reduction of microbial concentration.
Formulation of the sodium salt of Compound I and Methylparaban

Microorganism	6 Hours	24 Hours	7 Days	14 Days
Aspergillus niger	-	-	5.06*	5.06
Candida albicans	-	-	5.17*	5.17
E. coli	-	-	5.54*	5.54
Ps. aeruginosa	5.16	5.16	5.16*	5.16
S. aureus	0.10	0.10	5.12*	5.12

*Corresponds to "no recovery" of microorganisms.

Example 6.

AET of the sodium salt of Compound I, Methylparaben (1.8 mg/mL) and Propylparaben (0.2 mg/mL).

AET, according to EP and USP Procedures, were conducted on a solution containing the sodium salt of Compound I (80 mg/mL), methylparaben (1.8 mg/mL) and propylparaben (0.2 mg/mL) in 20 mM citrate buffer. The results of AET are provided in Table 10. The formulation satisfied USP criteria (see Table 14 for acceptance criteria) for all microorganisms. Furthermore, the formulation satisfied EP criteria A for all microorganisms, except for *S. aureus* at 6 and 24 hours time points.

TABLE 10. Log reduction of microbial concentration. Formulation of the sodium salt of Compound 1, Methylparaban and propylparaben.

Microorganism	6 Hours	24 Hours	7 Days	14 Days
Aspergillus niger	-	-	5.06	5.06
Candida albicans	-	-	5.17	5.17
E. coli	-	-	5.54	5.54
Ps. aeruginosa	5.16	5.16	5.16	5.16
S. aureus	0.06	0.24	5.12	5.12

Example 7.

AET of the sodium salt of Compound I and *m*-cresol (3 mg/ml).

AET, performed according to EP and USP Procedures, were conducted on a solution of the sodium salt of Compound I (80 mg/mL) and *m*-cresol (3 mg/mL) prepared by reconstitution of a lyophilized cake of the sodium salt of Compound I with Bacteriostatic Water containing 3mg/mL *m*-cresol. The results of AET are provided in Table 11. The formulation satisfied USP criteria (see Table 14 for acceptance criteria) for all microorganisms. Furthermore, the formulation satisfied EP criteria A for all microorganisms, except for *S. aureus* at 6 hours time point.

TABLE 11. Log reduction of microbial concentration. Formulation of the sodium salt of Compound I and *m*-cresol.

Microorganism	6 Hours	24 Hours	7 Days
Aspergillus niger	-	-	4.82*
Candida albicans	-	-	5.24*
E. coli	-	-	5.41*
Ps. aeruginosa	3.35	4.76	5.36*
S. aureus	1.56	4.76	5.61*

*Corresponds to "no recovery" of microorganisms.

Example 8

AET of the sodium salt of Compound I, methylparaben, propylparaben and benzyl alcohol.

AET, performed according to EP and USP Procedures, were conducted on a solution of the sodium salt of Compound I (80 mg/mL), methylparaben (1.8 mg/mL), propylparaben (0.2 mg/mL) and benzyl alcohol (8.6 mg/mL) and 20mM of citrate buffer, prepared by reconstitution of a lyophilized cake of the sodium salt of Compound I, methylparaben and propylparaben with bacteriostatic water for injection containing at least 9 mg/mL benyzl alcohol. Methylparaben and propylparaben were included in the lyophilized cake whereas benzyl alcohol was added with bacteriostatic water for injection. The results of AET are provided in Table 12. The formulation satisfied USP criteria (see Table 14 for acceptance criteria) for all microorganisms. Furthermore, the formulation satisfied EP criteria A for all microorganisms.

TABLE 12. Log reduction of microbial concentration. Formulation of the sodium salt of Compound I, methylparaben, propylparaben and benzyl alcohol.

Microorganism	6 Hours	24 Hours	7 Days
Aspergillus niger	-	-	4.82
Candida albicans	-	-	5.24
E. coli	-	-	5.41
Ps. aeruginosa	5.36	5.36	5.36
S. aureus	2.71	5.61	5.61

Example 9

AEP of the sodium salt of Compound I, *m*-cresol and methylparaben.

AET, performed according to EP Procedure, were conducted on a solution of the sodium salt of Compound I (80 mg/mL), methylparaben (1.8 mg/mL) and *m*-cresol (3 mg/mL), prepared by reconstitution of a lyophilized cake of the sodium salt of Compound I with bacteriostatic water for injection containing methylparaben and *m*-cresol. The results of AET are provided in Table 13. The formulation satisfied EP Criteria A (see Table 14 for acceptance criteria) for *S. auereus.*

TABLE 13. Log reduction of microbial concentration.
Formulation of the sodium salt of Compound I, *m*-cresol and methylparaben.

Microorganism	6 Hours	24 Hours
S. aureus	4.05	5.20

TABLE 14. AET Acceptance Criteria (USP and EP)

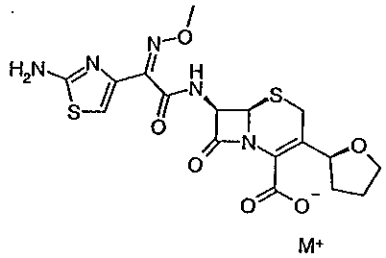
Microorganism	Criteria	Log Reduction				
		6 Hours	24 Hours	7 Days	14 Days	28 Days
Bacteria	EP criteria A	2	3	-	-	NR
	EP criteria B	-	1	3	-	NI
	USP cat 1A	-	-	1.0	3.0	NI
Fungi	EP criteria A	-	-	2	-	NI
	EP criteria B	-	-	-	1	NI
	USP cat 1A	-	-	NI	NI	NI

NR = no recovery; NI = no increase

European Pharmacopoeia, 3d edition, Supplement 2001, Council of Europe, Strasbourg; U.S. Pharmacopeia, and National Formulary USP24 NF19, 2000, United State Pharmacopeial Convention Inc., Rockville, MD.

CLAIMS:

1. A lyophilized pharmaceutical composition comprising a compound of Formula I,



I

M+

wherein M^+ is Na^+, Li^+ or K^+ and a preservative selected from methyl paraben, propyl paraben and a mixture of methyl and propyl parabens, wherein the composition has a solution pH in the range of 6.0 to 7.5.

2. A pharmaceutical composition according to claim 1, wherein M^+ is Na^+.

3. A pharmaceutical composition according to claim 1 or 2, further comprising a pharmaceutically acceptable buffer.

4. A pharmaceutical composition according to any one of claims 1 to 3, wherein the compound of Formula I is amorphous (6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino) acetyl]amino]-8-oxo-3-[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt.

5. A pharmaceutical composition according to claim 3 or 4, wherein the buffer is citrate.

6. A pharmaceutical composition according to any one of claims 1 to 5, further comprising a pharmaceutically acceptable bulking agent.

7. A pharmaceutical composition according to claim 6, wherein the bulking agent is selected from sugars, polyalcohols, amino acids, polymers, polysaccharides or inorganic salts.

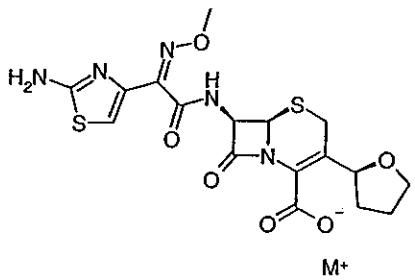
8. A pharmaceutical composition according to claim 7, wherein the sugars are selected glucose, maltose, sucrose or lactose; the polyalcohols are sorbitol or mannitol; the amino acid is glycine; the polymer is

polyvinylpyrrolidone; the polysaccharide is dextran; and the inorganic salts are sodium or potassium phosphates or sodium chloride.

9. A pharmaceutical composition according to any one of claims 1 to 8 wherein the pharmaceutically acceptable preservative is a combination of methyl and propyl parabens.

10. A kit comprising

a therapeutically effective amount of a lyophilized pharmaceutical composition comprising a compound of Formula I;



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wherein M^+ is Na^+, Li^+ or K^+ and a preservative selected from methyl paraben, propyl paraben and a mixture of methyl and propyl parabens, wherein the composition has a solution pH in the range of 6.0 to 7.5;

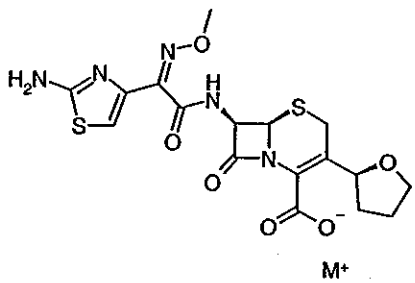
an aqueous pharmaceutically acceptable diluent; and

a first and second container means for containing the composition (a) and the diluent (b), wherein the first container is adapted to receive the diluent from the second container.

11. The kit according to claim 10, wherein M^+ is Na^+.

12. The kit according to claim 11 further comprising a pharmaceutically acceptable buffer.

13. Use of a compound of Formula (I)



I

wherein M^+ is Na^+, Li^+ or K^+ in the manufacture of a medicament for treating or preventing a condition caused by a bacterial infection in dogs and cats.

14. The use according to claim 13, wherein M^+ is Na^+.

15. The use according to claim 13 or 14, wherein the compound of Formula I is amorphous (6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)(methoxyimino) acetyl]amino]-8-oxo-3-[(2*S*)-tetrahydro-2-furanyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monosodium salt.

16. Use of a composition according to any one of claims 1 to 9 in the manufacture of a medicament for treating or preventing a condition caused by a bacterial infection in dogs and cats.

17. The use according to any one of claims 13 to 16, wherein the condition is a skin, soft tissue or urinary tract bacterial infection.

18. The use according to any one of claims 13 to 17, wherein the condition or infection is caused by or complicated by Gram positive or Gram negative bacteria.