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** CONTINUING DATA **********

** FOREIGN APPLICATIONS **********

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35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance

Verified and Acknowledged _____ Examiner's Signature Initials

ADDRESS
35743

TITLE
SINCALIDE FORMULATIONS

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Guy Beardsley
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Signature of person mailing correspondence

UTILITY PATENT APPLICATION TRANSMITTAL UNDER 37 C.F.R. § 1.53(b)	
Attorney Docket Number	50203/017001
Applicant	Edmund C. Metcalfe, Jo Anna Monteferrante, Margaret Newborn, Irene Ropiak, Ernst Schramm, Gregory W. White, and Julius P. Zodda
Title	SINCALIDE FORMULATIONS
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None	
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□ Applicant claims small entity status under 37 C.F.R. § 1.27.	
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■ Enclosed is a check for $2828.00 to cover the total fees.
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August 16, 2002
Date

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APPLICATION

FOR

UNITED STATES LETTERS PATENT

APPLICANT : Edmund C. Metcalfe, Jo Anna Monteferrante, Margaret Newborn, Irene Ropiak, Ernst Schramm, Gregory W. White, Julius P. Zodda

TITLE : SINCALIDE FORMULATIONS

F:\50203\50203.017001---Utility Cover Sheet.wpd

SINCALIDE FORMULATIONS

Field of the Invention

The invention relates to pharmaceutically acceptable formulations of sincalide.

Background of the Invention

KINEVAC® (Sincalide for Injection, USP) is a cholecystopancreatic-gastrointestinal hormone peptide for parenteral administration. The active pharmaceutical ingredient, 1-De(5-oxo-L-glutamine-5-L-proline)-2-de-L-methioninecaerulein or "sincalide" (CAS# 25126-32-3), is a synthetically prepared C-terminal octapeptide of cholecystokinin (CCK-8), with the following amino acid sequence: Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2.

KINEVAC® was first introduced in 1976, and was finished as a sterile, nonpyrogenic, lyophilized white powder in a 5-mL (nominal) glass vial to contain: 5 µg sincalide with 45 mg sodium chloride to provide tonicity; sodium hydroxide or hydrochloric acid may have been added for pH adjustment (pH 5.5 - 6.5). The type I glass vial was sealed under a nitrogen headspace with a Tompkins B0849 closure. This two-ingredient formulation was incorporated into the U.S. Pharmacopea/National Formulary, USP 24, NF 19, January 1, 2000.

Since its introduction, various drawbacks in the manufacturing and analysis of KINEVAC® have been identified. For example, the two-ingredient formulation suffers from potency variability. This variability was exacerbated by the fact that the formulation was analyzed using a guinea pig gallbladder contraction bioassay for potency of both sincalide and KINEVAC®. This bioassay was unable to distinguish between bioactivity of sincalide and bioactivity of sincalide degradants. Accordingly, a 20% overage of sincalide was required in previous sincalide formulations to compensate for the limitations of the bioassay. Thus, there is a need for sincalide formulations having improved and consistent potency as established by a sincalide specific assay such as HPLC.

Summary of the Invention

The present invention satisfies the need for improved sincalide formulations by providing formulations that eliminate the need for a 20% overage of sincalide. The sincalide formulations of the invention are also purer than prior art formulations, and have fewer degradants and more consistent potency. In addition, the purity of these formulations may be assessed by HPLC, thus eliminating the need for the bioassay of the prior art formulations.

The present invention provides sincalide formulations adapted for administration by injection. These sincalide formulations are characterized by improved stability and may be prepared as a relatively large volume batch (≈100 L).

In one aspect, the invention features sincalide formulations that include an effective amount of sincalide, a bulking agent/tonicity adjuster, one or more stabilizers, a surfactant, a chelator, and a buffer. The invention also features kits and methods for preparing improved sincalide formulations, as well as methods for treating, preventing, and diagnosing gall bladder-related disorders using sincalide formulations.

The formulations of the invention preferably have a pH between 6.0 and 8.0. Suitable buffers include, but are not limited to, phosphate, citrate, sulfosalicylate, borate, acetate and amino acid buffers. Phosphate buffers, such as dibasic potassium phosphate, are preferred.

In various embodiments of the invention, the surfactant is a nonionic surfactant, preferably a polysorbate, such as polysorbate 20 or polysorbate 80; the chelator is pentetic acid (DTPA); and the stabilizer is an antioxidant and/or amino acid. In a particularly desirable embodiment of the invention, the formulation includes a plurality of stabilizers, preferably L-arginine monohydrochloride, L-methionine, L-lysine monohydrochloride, and sodium metabisulfite.

Suitable bulking agents/tonicity adjusters include, but are not limited to, mannitol, lactose, sodium chloride, maltose, sucrose, PEG's, cyclodextrins, dextran, polysucrose

The sincalide formulations of the invention may also be administered to patients receiving total parenteral nutrition (TPN), in order to treat and/or prevent TPN-related disorders.

Other features and advantages of the invention will be apparent from the following detailed description thereof and from the claims.

Brief Description of the Drawings

FIG. 1 is a drawing illustrating the chemical structure of 1-De(5-oxo-L-glutamine-5-L-methioninecaerulein or "sincalide" (CAS# 25126-32-3). The amino acid residues "Met 3" and "Met 6" are outlined by dashed lines.

FIG. 2 is a drawing illustrating the chemical structure of sincalide (Met 3) monosulfoxide.

FIG. 3 is a drawing illustrating the chemical structure of sincalide (Met 6) monosulfoxide.

FIG. 4 is a drawing illustrating the chemical structure of sincalide (Met 3, 6) disulfoxide.

FIG. 5 is a graphical representation of the effect of pH on the recovery of sincalide in 35 mM phosphate buffer over 24 hours. At each pH for which data is shown, the bars represent 0, 6, and 24 hours, from left to right.

FIG. 6 is a graphical representation of the effect of pH on the recovery of sincalide in a formulation of the invention over 8 hours. At each pH for which data is shown, the bars represent 0, 4, and 8 hours, from left to right.

FIG. 7 is a graphical representation of the percent sincalide Met 3 and Met 6 monosulfoxides (vs sincalide), in the presence and absence of pentetic acid (DTPA).

FIG. 8 is a chromatogram of KINEVAC® experimental formulation (no DTPA) spiked with 0.63mM Cu^{2+}.

FIG. 9 is a chromatogram of KINEVAC® experimental formulation (1mM DTPA) spiked with 0.63mM Cu^{2+}.

(Ficoll), and polyvinylpyrrolidine (PVP). D-Mannitol is a preferred bulking agent/tonicity adjuster.

In a particularly preferred embodiment, the reconstituted formulation includes 0.0008 to 0.0012 mg/mL active ingredient (i.e., sincalide); 20.0 to 50.0 mg/mL mannitol, 2.0 to 7.0 mg/mL arginine; 0.2 to 1.0 mg/mL methionine; 2.0 to 30.0 mg/mL lysine; 0.002 to 0.012 mg/mL sodium metabisulfite; 0.000001 to 0.003 mg/mL polysorbate 20, 0.1 to 3.0 mg/mL pentetic acid (DTPA); and 5.4 to 12.0 mg/mL potassium phosphate (dibasic). In a more preferred embodiment, the reconstituted formulation includes about 0.001 mg/mL sincalide; about 34 mg/mL D-mannitol, about 6 mg/mL L-arginine monohydrochloride; about 0.8 mg/mL L-methionine; about 3 mg/mL L-lysine monohydrochloride; about 0.008 mg/mL sodium metabisulfite; less than about 0.01 mg/mL polysorbate 20, about 0.4 mg/mL pentetic acid (DTPA); and about 1.8 mg/mL potassium phosphate (dibasic).

The kits of the invention may, for example, include the various components of the formulation as a mixture in powder form, along with a container (e.g., a vial) to hold the powder mixture and a physiologically acceptable fluid for reconstitution of the formulation. The components of the formulation may be present in the kit either in the powder mixture or in the fluid portion. Kits of the invention may also include all components in a liquid mixture or some components in a liquid form and some in the form of a powder.

The formulations of the invention have improved stability and potency compared to previous sincalide formulations, and are useful as diagnostic aids for imaging the hepatobiliary system of a patient. When used as a diagnostic aid, the sincalide formulations may, for example, be co-administered with a radiopharmaceutical agent having rapid hepatic uptake, such as ^{99m}Tc-mebrofenin, or similar hepatobiliary imaging agents, to assist in the diagnosis of gallbladder diseases and related disorders. Additionally, the formulations may be administered before and/or after diagnostic imaging (including for example, magnetic resonance imaging, scintigraphic imaging, ultrasound imaging, etc.)

FIG. 10 is a chromatogram of KINEVAC® experimental formulation (no DTPA) spiked with 0.18mM Mn^{2+}.

FIG. 11 is a chromatogram of KINEVAC® experimental formulation (1mM DTPA) spiked with 0.18mM Mn^{2+}.

FIG. 12 shows representative full-scale and expanded scale chromatograms of a lyophilized reformulation of KINEVAC® upon reconstitution with 5mL water, resulting in a sincalide concentration of 1μg/mL.

Detailed Description of the Invention

In order to develop an improved sincalide formulation a series of studies, described in the Examples below, were conducted to determine the effects of various excipients on formulations of sincalide. Through these studies, we discovered that the potency and stability of sincalide formulations can be significantly enhanced through the careful selection of excipients that provide certain desired functions. Accordingly, the present invention provides novel sincalide formulations having improved stability and/or potency over previous formulations.

As used herein, the term "sincalide" includes the synthetically-prepared C-terminal octapeptide of cholecystokinin (CCK-8), with the amino acid sequence: Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2, as well as derivatives thereof which have been optimized or modified (to improve stability, potency, pharmacokinetics, etc.), but retain the biological activity of the original octapeptide. For example, peptides in which the methionine and/or aspartic acid residues have been replaced without significantly affecting the biological activity are included within "sincalide" as the term is used herein. Similarly, the term "sincalide" encompasses not only monomeric, but multimeric forms of the peptide, as well as physiologically active degradants or portions of the peptide and its derivatives.

The sincalide formulations of the invention can include a variety of excipients, such as, for example, antioxidants, buffers, bulking agents/tonicity adjusters, chelating agents, complexing agents, crosslinking agents, co-solvents, osmolality adjustors,

solubilizers, surfactants, stabilizers, pH adjustors, lyoprotectants/cryoprotectants, air/liquid and/or ice-liquid interface protectants (protectants against surface induced denaturation), freeze-thaw protectants, protectants against protein/peptide denaturation, protectants for rehydration, and wetting agents. In preferred embodiments, the formulations include excipients that perform the functions of at least: (i) a bulking agent/tonicity adjuster, (ii) a stabilizer, (iii) a surfactant, (iv) a chelator, and (v) a buffer. Typically, each of these functions is performed by a different excipient. However, in some embodiments of the invention a single excipient may perform more than one function. For example, a single excipient may be multi-functional, e.g. amino acids may function as bulking agents, stabilizers and/or buffers and other excipients may function, for example, as both a stabilizer and a chelator or as both a bulking agent and a tonicity adjuster. Alternatively, multiple excipients serving the same function may be used. For example, the formulation may contain more than one excipient that functions as a stabilizer.

Table 1 below shows the concentration ranges for various excipients that were investigated. In general, the range studies were based on a 2-mL fill of bulk solution per vial before lyophilization. After reconstitution with 5 mL of water for injection the final sincalide formulation results in an isotonic solution. The concentration ranges of the various ingredients provided in Table 1 can be adjusted upward or downward, if necessary in conjunction with: increasing or decreasing the fill volume per vial, obtaining the desired pH, obtaining the desired reconstitution volume, and the desirability of achieving tonicity in the final reconstituted solution. For example, as indicated above, the concentrations provided in Table 1 were developed to provide an isotonic solution; however, one skilled in the art would recognize that a broader range of concentrations could be used if an isotonic solution was not required.

Table 1. Concentration ranges for excipients for preferred sincalide formulations.

Excipient	Function	Range (mg/mL Bulk)	Range (mg/vial)	Range (mg per 1mL after reconst)	Final Formulation (mg)		
					1mL Bulk	1 vial Target	1 mL after reconst.
(Sincalide)	Active Ingredient	0.0025	0.0050	**0.0008-0.0012**	0.0025	0.0050	0.0010
Mannitol	Bulking Agent/Cake Forming Agent/Tonicity Adjuster	50.0 – 125.0	100-250	**20.0-50.0**	85	170	34
TWEEN®-20	Non-Ionic Surfactant/Solubilizing Agent/Wetting Agent	0.0000025-0.0075	0.0000050 -0.0150	**0.0000010-0.0030**	< 0.01	< 0.01	< 0.01
DTPA	Chelator/Stabilizer/Antioxidant/ Complexing Agent/Preservative/pH Adjuster	1.0	2.0	**0.1-3.0**	1.0	2.0	0.4
Sodium Metabisulfite	Antioxidant/Preservative/ Stabilizer	0.005 – 0.030	0.010-0.060	**0.002-0.012**	0.020	0.040	0.008
Potassium Phosphate, dibasic	Buffer/pH Adjuster/Dissolution Aid	2.7 – 4.5	5.4-12.0	**1.1-1.8**	4.5	9.0	1.8
Potassium Phosphate, monobasic	Buffer/pH Adjuster/Dissolution Aid	1.0 – 6.5	9.6-13.0	**1.92-2.6**	0	0	0
Methionine	Stabilizer	0.5 – 2.5	1.0-5.0	**0.2-1.0**	2.0	4.0	0.8
Lysine	Stabilizer/Lyoprotectant/ Cryoprotectant	5.0 – 30.0	10.0-60.0	**2.0-30.0**	7.5	15.0	3.0
Arginine	Stabilizer/Lyoprotectant/ Cryoprotectant/pH Adjuster	5.0 –17.5	10.0-35.0	**2.0-7.0**	15	30.0	6.0
Sodium Chloride	Tonicity Adjuster	4.5 – 9.0	9.0-18.0	**1.8-3.6**	0	0	0

Alternative excipients include TWEEN®-80, potassium metabisulfite, sodium phosphate dibasic, sodium phosphate monobasic, and potassium chloride. Additional alternatives are listed below.

Table 2 shows preferred ranges for preferred excipients in the bulk solutions, vials and after reconstitution. All concentrations shown for the bulk solution are based on a 2 mL fill volume. The ingredient quantities are matched to result in a pH slightly below neutral and result in an isotonic solution after reconstitution of the lyophilized vial as indicated by an osmolality in the range of 180 to 320 mOsm/kg , preferably, 240 to 320 mOsm. The columns titled "Final Formulation" represent particularly preferred formulations.

Table 2. Osmolality values for various sincalide formulations.
(All formulations contain 0.0025mg CCK-8/mL.; "dibasic" and "monobasic" refer to dibasic and monobasic potassium phosphate; "Na meta" refers to sodium metabisulfite)

Formulation Excipients (mg/mL Bulk)	Calculated mOsm/kg
Mannitol (125.0)	292
Dibasic (3.75)	
DTPA (1.0)	
Mannitol (95.0)	244
Dibasic (4.0)	
Monobasic (2.8)	
DTPA(1.0)	
Mannitol (103.0)	244
Dibasic (3.75)	
DTPA (1.0)	
Mannitol (75.0)	244
NaCl (4.5)	
Dibasic (3.75)	
DTPA (1.0)	
Mannitol (85.0)	187
TWEEN® 20 (0.005)	
Dibasic (2.75)	
DTPA (1.0)	
Methionine (2.0)	
Lysine (15.0)	
Mannitol (50.0)	247
NaCl (9.0)	
Dibasic (3.00)	
DTPA (1.0)	

TWEEN® 20 (0.0075)	264
Mannitol (75.0)	
KCl (6.0)	
Dibasic (3.25)	
Monobasic (1.0)	
DTPA (1.0)	
Methionine (2.0)	
TWEEN® 20 (0.005)	264
Mannitol (75.0)	
KCl (6.0)	
Dibasic (3.25)	
Monobasic (1.0)	
DTPA (1.0)	
Methionine (2.0)	
TWEEN® 20 (0.0025)	264
Mannitol (75.0)	
KCl (6.0)	
Dibasic (3.25)	
Monobasic (1.0)	
DTPA (1.0)	
Methionine (2.0)	
TWEEN® 20 (2.5ng)	314
Mannitol (85.0)	
Dibasic (4.50)	
DTPA (1.0)	
Na metabisulfite (0.020)	
Methionine (2.0)	
Lysine (7.50)	
Arginine (15.0)	
Na Meta (0.015)	257
Mannitol (85.0)	
Dibasic (2.75)	
DTPA (1.0)	
20 (0.005)	
Methionine (2.0)	
Lysine (7.50)	
Arginine (15.0)	

9

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Na Meta (0.030) **Mannitol (85.0)** Dibasic (2.75) DTPA (1.0) TWEEN® 20 (0.005) Methionine (2.0) Lysine (7.50) Arginine (15.0)	257
Na Meta (0.005) **Mannitol (85.0)** Dibasic (2.75) DTPA (1.0) TWEEN® 20 (0.005) Methionine (2.0) Lysine (7.50) Arginine (15.0)	257
Na Meta (0.020) **Mannitol (85.0)** Dibasic (3.00) DTPA (1.0) TWEEN® 20 (0.005) Methionine (2.0) Lysine (7.50) Arginine (15.0)	259
Dibasic (2.75) **Mannitol (85.0)** Na Meta (0.015) DTPA (1.0) TWEEN® 20 (0.005) Methionine (2.0) Lysine (7.50) Arginine (15.0)	257
Dibasic (3.00) **Mannitol (85.0)** Na Meta (0.020) DTPA (1.0) TWEEN® 20 (0.005) Methionine (2.0) Lysine (7.50) Arginine (15.0)	259

Dibasic (3.25) **Mannitol (75.0)** KCl (6.0) TWEEN® 20 (0.0025) Monobasic (1.0) DTPA (1.0) Methionine (2.0)	264
Dibasic (4.50) **Mannitol (85.0)** TWEEN® 20 (2.5ng) DTPA (1.0) Na metabisulfite (0.020) Methionine (2.0) Lysine (7.50) Arginine (15.0)	314
Methionine (2.0) **Mannitol (75.0)** NaCl (5.0) TWEEN® 80 (0.025) Monobasic (1.0) DTPA (1.0) Dibasic (3.25)	262
Methionine (1.5) **Mannitol (75.0)** NaCl (5.0) TWEEN® 80 (0.025) Monobasic (1.0) DTPA (1.0) Dibasic (3.25)	262
Methionine (1.0) **Mannitol (75.0)** NaCl (5.0) TWEEN® 80 (0.025) Monobasic (1.0) DTPA (1.0) Dibasic (3.25)	262
Methionine (0.5) **Mannitol (75.0)** NaCl (5.0) TWEEN® 80 (0.025) Monobasic (1.0) DTPA (1.0) Dibasic (3.25)	262

11

17

Methionine (2.5) **Mannitol (75.0)** NaCl (5.0) TWEEN® 80 (0.005) Monobasic (1.0) DTPA (1.0) Dibasic (3.25)	262
Lysine (5.0) **Mannitol (95.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0)	209
Lysine (15.0) **Mannitol (85.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0)	187
Lysine (30.0) **Mannitol (70.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0)	245
Arginine (17.5) **Mannitol (85.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0)	245
Arginine (10.0) **Mannitol (85.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0)	232
Arginine (5.0) **Mannitol (85.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0) Lysine (7.5)	238

12

12

Arginine (8.75) **Mannitol (85.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0) Lysine (7.5)	245
Arginine (15.0) **Mannitol (85.0)** TWEEN® 20 (0.005) Dibasic (2.75) DTPA (1.0) Methionine (2.0) Lysine (7.5)	257

Chelators

Excipient impurities and/or stopper extractables can introduce trace metals into pharmaceutical formulations. Sincalide contains two methionine residues (Met 3 and Met 6) that are susceptible to oxidation by free metals. Thus, the sincalide formulations of the invention contain chelators to inhibit the oxidation of the two methionine residues present in sincalide (Met 3 and Met 6). Preferred chelators include pentetic acid (DTPA), edetic acid (EDTA) and derivatives thereof, including salts. DTPA is a preferred chelator. As described in Example 2 below, the amounts of the degradants, sincalide Met 3 and sincalide Met 6 monosulfoxides, increase in the presence of certain metals and in the absence of DTPA, while the presence of DPTA has an inhibitory effect on the formation of these monosulfoxides. In particular, copper and manganese, in the absence of DTPA, have the greatest oxidative effect on the methionine residues of sincalide resulting in combined height percentages of Met 3 and Met 6 monosulfoxides (vs sincalide) of 85.5 and 128.9, respectively.

In a preferred embodiment, the sincalide formulations contain between 0.1 and 3.0 mg of DTPA per mL after reconstitution. In a particularly preferred embodiment, sincalide formulations of the invention contain 0.4 mg DTPA/mL after reconstitution with 5mL.

Buffering Agents

Buffering agents are employed to stabilize the pH of sincalide formulations of the invention, and consequently, reduce the risk of chemical stability at extreme pH values. Buffering agents useful in the preparation of formulation kits of the invention include, but are not limited to, phosphoric acid, phosphate (e.g. monobasic or dibasic sodium phosphate, monobasic or dibasic potassium phosphate, etc.), citric acid, citrate (e.g. sodium citrate, etc.), sulfosalicylate, acetic acid, acetate (e.g. potassium acetate, sodium acetate, etc.), methyl boronic acid, boronate, disodium succinate hexahydrate, amino acids, including amino acid salts (such as histidine, glycine, lysine, imidazole), lactic acid, lactate (e.g. sodium lactate, etc.), maleic acid, maleate, potassium chloride, benzoic acid, sodium benzoate, carbonic acid, carbonate (e.g. sodium carbonate, etc.), bicarbonate (e.g. sodium bicarbonate, etc.), boric acid, sodium borate, sodium chloride, succinic acid, succinate (e.g. sodium succinate), tartaric acid, tartrate (e.g. sodium tartrate, etc.), tris-(hydroxymethyl)aminomethane, biological buffers (such as N-2-hydroxyethylpiperazine,N'-2-ethanesulfonic acid (HEPES), CHAPS and other "Good's" buffers), and the like.

Phosphate is a preferred buffering agent due to its lack of interaction with sincalide and an ideal buffering capacity in the physiological pH range. Dibasic potassium phosphate is a particularly preferred buffer in sincalide formulations of the invention. As described in Example 1 below, a sincalide formulation of the invention proved to be stable over a pH range of 5.5 –9.1. Within the pH range of 5.5 –8.5, no distinct pH-dependent related trends in initial sincalide recovery were observed with a sincalide formulation of the invention. Preferably, a sincalide formulation of the invention has a pH from 6.0 to 8.0.

Stabilizers

The octapeptide, sincalide, contains one tryptophan and two methionine residues. Methionine has been identified as one of the most easily oxidizable amino acids, which

degrades to its corresponding sulfoxide and, under more strenuous oxidation conditions, its sulfone. The mechanisms of oxidation appear to be highly dependent on the reactive oxygen species under consideration: peroxide, peroxyl radicals, singlet oxygen, and hydroxyl radical have all been shown to oxidize methionine residues to sulfoxides and other products. Therefore, based on the potential for oxidation of this peptide, it was necessary to identify functional additives for peptide stabilization.

Antioxidants/Reducing Agents. In a preferred embodiment of the invention, the sincalide formulation contains an antioxidant or reducing agent as a stabilizer. A wide variety of antioxidants or reducing agents can be used as stabilizers, including but not limited to, acetylcysteine, cysteine, ascorbic acid, benzyl alcohol, citric acid, pentetic acid or diethylenetriamine pentaacetic acid (DTPA), propyl gallate, methylparaben, sulfoxylate, propylparaben, edetic acid or ethylenediaminetetraacetic acid (EDTA), disodium EDTA dihydrate, dithiothreitol, glutathione, monothioglycerol, potassium metabisulfite, sodium formaldehyde sulfoxylate, sodium sulfite, sodium succinate, sodium metabisulfite, stannous chloride, thioacetic acid, thiodiglycerol, thioethanolamine, thioglycolic acid, 2-aminoethanethiol (cysteamine), butylated hydroxyanisole (BHT), and sodium sulfate and derivatives thereof, including salts and sulfurous acid salts. Sodium metabisulfite is a preferred antioxidant stabilizer. Additionally, DTPA, which is a preferred chelator, also may be an antioxidant stabilizer.

Amino Acids. Amino acids have also been used as stabilizers or co-stabilizers of peptides to: act as cryoprotectants during freeze drying, stabilize against heat denaturation, inhibit aggregate formation, improve solubility or rehydration, inhibit isomerization, reduce surface adsorption, or act as chelating agents. They can also increase the product glass transition temperature (T_g) and thereby increase process stability, as well as stabilize the product by minimizing overdrying during secondary drying. Surface exposed residues can react readily with oxidizing agents at physiological pH, scavenging oxidizing molecules and protecting critical regions of peptides.

Various D- and/or L-amino acids can be used as stabilizers in sincalide formulations. As used herein "amino acid(s)"and the names of specific amino acids (e.g

arginine, lysine, methionine, etc.) encompass D- and/or L-amino acids, amino acid salts, derivatives, homologs, dimers, oligomers, or mixtures thereof. Preferred amino acids for use as stabilizers in the present invention include methionine, lysine, and arginine. Examples of other amino acids (and amino acid salts) suitable as stabilizers include, but are not limited to, arginine glutamate, asparagine, gamma aminobutyric acid, glycine (and glycine buffer), glutamic acid, glutamate, sodium glutamate, histidine (and histidine buffer), lysine glutamate, lysine aspartate, arginine aspartate, imidazole, serine, threonine, alanine, polyglutamic acid, polylysine, glycylglycine and the like, including hydroxypropyl and galactose derivatives. In one particularly preferred embodiment, L-arginine monohydrochloride, L-methionine and L-lysine monohydrochloride are used.

Cryoprotectants/Lyoprotectants

Various cryoprotectants/lyoprotectants can be used in the present invention. Suitable cryoprotectants structure water molecules such that the freezing point is reduced and/or the rate of cooling necessary to achieve the vitreous phase is reduced. They also raise the glass transition temperature range of the vitreous state. These include, but are not limited to: dimethylsulfoxide (DMSO), dextran, sucrose, 1,2-propanediol, amino acids/salts such as, glycine, lysine, arginine, aspartic acid, histidine, proline, etc., glycerol, sorbitol, sodium chloride, fructose, trehalose, raffinose, stachychose, propylene glycol, 2,3- butanediol, hydroxyethyl starch, polyvinylpyrrolidone (PVP), PEG's and similar compounds, protein stabilizers, such as human serum albumin, bovine serum albumin, bovine gamma globulin, gelatin (or derivatives, such as Prionex, etc.), dextrose, glucose, maltose, arabinose, lactose, inositol, polyols (such as sorbitol, xylitol, erithritol, glycerol, ethylene glycol, etc.), tetramethylglucose, sodium sulfate, cyclodextrins and combinations thereof. Lysine and arginine are preferred cryoprotectants/lyoprotectants.

Surfactants/Solubilizers/Surface Active Agents

Peptides are susceptible to physical degradation through denaturation, aggregation, precipitation, container surface adsorption and/or agitation induced denaturation. The

addition of a nonionic surfactant, such as polysorbate, to the formulation, may reduce the interfacial tension or aid in solubilization thus preventing or reducing denaturation and/or degradation at air/liquid or liquid/solid interfaces of the product in solution.

Surfactants/solubilizers include compounds such as free fatty acids, esters of fatty acids with polyoxyalkylene compounds like polyoxypropylene glycol and polyoxyethylene glycol; ethers of fatty alcohols with polyoxyalkylene glycols; esters of fatty acids with polyoxyalkylated sorbitan; soaps; glycerol-polyalkylene stearate; glycerol-polyoxyethylene ricinoleate; homo- and copolymers of polyalkylene glycols; polyethoxylated soya-oil and castor oil as well as hydrogenated derivatives; ethers and esters of sucrose or other carbohydrates with fatty acids, fatty alcohols, these being optionally polyoxyalkylated; mono- , di- and triglycerides of saturated or unsaturated fatty acids; glycerides or soya-oil and sucrose; sodium caprolate, ammonium sulfate, sodium dodecyl sulfate (SDS), Triton-100 and anionic surfactants containing alkyl, aryl or heterocyclic structures.

Examples of preferred surfactants/solubilizers for use in the present invention include, but are not limited to, pluronics (e.g., Lutrol F68, Lutrol F127), Poloxamers, SDS, Triton-100, polysorbates such as TWEEN® 20 and TWEEN® 80, propylene glycol, PEG and similar compounds, Brij58 (polyoxyethylene 20 cetyl ether), cremophor EL, cetyl trimethylammonium bromide (CTAB), dimethylacetamide (DMA), NP- 40 (Nonidet P-40), and N-methyl-2-pyrrolidone (Pharmasolve), glycine and other amino acids/amino acid salts and anionic surfactants containing alkyl, aryl or heterocyclic structures, and cyclodextrins. TWEEN® 20 is the most preferred surfactant in formulations of the invention.

Bulking Agents/Tonicity Adjusters

Due to the small amount of sincalide present in the formulations of the invention, bulking agents/tonicity adjusters are useful to provide structure and support for the active ingredient, sincalide, as well as to provide tonicity. Bulking agents/tonicity adjusters (also called lyophilization aids) useful in the preparation of lyophilized products of the

invention are known in the art and include mannitol, lactose, potassium chloride, sodium chloride, maltose, sucrose, PEG's (such as, for example, PEG 300, PEG 400, PEG 3350, PEG 6000, PEG 8000 and the like, etc.), trehalose, raffinose, dextrose, polygalacturonic acid galacturonic acid, amino acids (including amino acid salts) such as lysine, arginine, glycine, galactose, etc.), cyclodextrins, such as hydroxypropyl-γ-cyclodextrin (HP-γ-CD), dextran, Ficoll, and polyvinylpyrrolidone (PVP). Of these, D-mannitol is the most preferred bulking agent/tonicity adjuster for use with the invention.

Other Excipients

Other excipients, which may optionally be used in the formulations of the invention include preservatives (e.g., benzalkonium chloride), osmolality adjusters (e.g., dextrose), lyoprotectants (e.g., sodium sulfate), solubilizers, tonicity adjusters (e.g. sodium chloride), cake forming agents, complexing agents, and dissolution aids. A listing of various excipients that can be used in sincalide formulations for parenteral administration can be found in, for example, The Handbook of Pharmaceutical Additives, Second Edition, edited by Michael & Irene Ash; Remington's Pharmaceutical Sciences, (18th Edition), edited by A. Gennaro, 1990, Mack Publishing Company, Easton, PA and Pollock et al.; Strickly, Robert G., Parenteral Formulations of Small Molecules Therapeutics Marketed in the United States (1999)-Part I, *PDA Journal of Pharmaceutical Science and Technology*, 53(6):324 (1999); Strickly, Robert G., Parenteral Formulations of Small Molecules Therapeutics Marketed in the United States (1999)-Part II, *PDA Journal of Pharmaceutical Science and Technology*, 54(1):69 (2000); Parenteral Formulations of Small Molecules Therapeutics Marketed in the United States (1999)-Part III, *PDA Journal of Pharmaceutical Science and Technology*, 54(2):154 (2000); Nema, Sandeep, et al., Excipients and Their Use in Injectable Products, *PDA Journal of Pharmaceutical Science and Technology*, 51(4):166 (1997); Wang, Y.J., et al., Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers (Technical Report No. 10), *Journal of Parenteral Science and Technology*, Vol.42 (2S), Supplement 1988; Carpenter, J. et al., Freezing- and Drying-Induced Perturbations of

Protein Structure and Mechanisms of Protein Protection by Stabilizing Additives, in *Drugs and The Pharmaceutical Sciences*, Louis Rey and Joan C. May., eds., Marcel Dekker, Inc. New York, NY (1999); Michael J. Pikal, Mechanisms of Protein Stabilization During Freeze-Drying and Storage: The Relative Importance of Thermodynamic Stabilization and Glassy State Relaxation Dynamics, in *Drugs and The Pharmaceutical Sciences*, Louis Rey and Joan C. May., eds., Marcel Dekker, Inc. New York, NY (1999); Shah, D., et al., The Effects of Various Excipients on the Unfolding of Basic Fibroblast Growth Factor, *PDA Journal of Pharmaceutical Science & Technology*, 52(5):238 (1998); Powell, M.F., et al., Compendium of Excipients for Parenteral Formulations, *PDA Journal of Pharmaceutical Science & Technology*, 52(5):238 (1998); and Inactive Ingredient Guide, Div. Of Drug Information Resources, FDA, CDER, Jan. 1996; Handbook of Injectable Drugs, Edition 8, Am. Soc. Hospital Pharmacists, 1994, L.A.Trissel.

Formulation Kits

Kits of the present invention preferably comprise one or more vials containing the sterile formulation of a predetermined amount of sincalide, a lyophilization aid or bulking agent/tonicity adjuster, one or more stabilizers, a surfactant, a chelator, and a buffer. The one or more vials that contain all or part of the formulation can independently be in the form of a sterile solution or a lyophilized solid. Buffering agents useful in the preparation of formulation kits of the invention are discussed herein and include, for example phosphate, citrate, sulfosalicylate, and acetate, and amino acids (including amino acid salts). Dibasic potassium phosphate is a preferred buffer in sincalide formulations of the invention. The kits may also include a fluid portion, for example water or saline, for reconstitution of the formulation prior to injection.

Lyophilization aids or bulking agent/tonicity adjusters useful in the preparation of lyophilized kits include those dicussed above, particularly, mannitol, lactose, sodium chloride, maltose, sucrose, PEG's, galaturonic acid, polygalcturonic acid, cyclodextrins, such as hydroxypropyl-γ-cyclodextrin (HP-γ-CD) and the like, dextran, amino acids

(including amino acid salts), Ficoll, and polyvinylpyrrolidone (PVP). Of these, mannitol, sodium chloride, maltose, sucrose, PEG's, HP-γ-CD, and dextran are preferred bulking agents/tonicity adjusters for use with the invention, with mannitol being the most preferred.

As discussed, a component in a formulation kit can also serve more than one function. For example, an excipient which serves as a stabilizer may also serve as the chelator and an excipient which serves as a bulking agent may also serve as a tonicity adjuster. In addition, in some embodiments, the excipients are all in dry powder form, or all in liquid form while in other embodiments, some of the excipients are in dry form and others are in a fluid portion included in or sold separately from the kit.

A particularly preferred kit of the invention contains: about 0.005 mg sincalide, about 170 mg D-mannitol, less than or equal to 0.01 mg TWEEN® 20, about 2 mg DTPA, about 0.04 mg sodium metabisulfite, about 9 mg potassium phosphate (dibasic) about 4 mg L-methionine, about 15 mg L-lysine monohydrochloride, and about 30 mg L-arginine monohydrochloride.

Therapeutic/Diagnostic Uses

Sincalide is a synthetic analog of the endogenously produced hormone cholecystokinin (CCK-8). CCK-8 acts on receptors within the gallbladder wall causing it to contract, cleaning out any remaining sludge or bile that may have accumulated within the gallbladder. CCK-8 increases bile flow and small and large bowel motility, causes the pyloric sphincter to contract and increases pancreatic enzyme secretion. CCK-8 also causes delayed biliary to bowel transit. Sincalide has a more rapid physiologic effect on the gallbladder in terms of contraction and relaxation than the endogenous hormone (CCK-8) produced by the body, making sincalide formulations useful as diagnostic aids for hepatobiliary imaging, when administered alone or in conjunction with a hepatobiliary imaging agent. For example, sincalide may be administered before and/or after diagnostic imaging (such as, for example, magnetic resonance imaging,

scintigraphic imaging, ultrasound imaging, etc.) to improve visualization and/or diagnosis of various disease states.

In one embodiment, hepatobiliary imaging can be performed using, for example, hepatobiliary scintigraphy, an instrumental imaging tool used in the diagnosis and evaluation of hepatobiliary disease. Detection of diseases, such as acute and chronic cholecystitis, biliary obstruction, bile leaks, and other forms of hepatobiliary disease, help the physician to better determine the appropriate course of treatment and management of the patient suffering from a suspected hepatobiliary pathology.

As explained below, the indications for use of sincalide in conjunction with hepatobiliary imaging include (a) pretreatment of patients who have not eaten for more than 20 to 24 hours prior to imaging (in order to empty the gallbadder (GB) of non-radiolabelled bile) and (b) use in the analysis of gallbladder motor function, including the determination of GBEF (gallbladder ejection fraction).

It is important to properly prepare the patient prior to hepatobiliary imagingin order to achieve high quality imaging and reduce the number of false positive and negative results. Preferably, patients should have nothing to eat for 4 to 12 hours prior to hepatobiliary imaging. Prolonged fasting, however, may result in false positive test results (i.e. failure to visualize the gallbladder). If a patient has not eaten for more than 24 hours, the patient is preferably pretreated with sincalide by administration of the sincalide formulation described herein prior to imaging. Typically, the gallbladder contracts within 15 minutes after sincalide injection and the hepatobiliary imaging agent (e.g., radiotracer) is injected 30 minutes later. The gallbladder is then emptied and is better able to take up and accumulate imaging agent (e.g., radiotracer), which helps to reduce the number of false positive studies.

The preferred radiopharmaceuticals used for hepatobiliary imaging include, but are not limited to, Tc 99m IDA (Iminodiacetic acid) analogs, such as Tc-99m mebrofenin (CHOLETEC®), Tc-99m disofenin (DISIDA), and Tc-99m lidofenin (see also U.S.

Patent No. 4,418,208). Tc-99m mebrofenin is a preferred hepatobiliary imaging agent. Methods for coadministration of Tc 99m IDA (Iminodiacetic acid) analogs with CCK and sincalide are known in the art and described in, for example, Ziessman HA., Cholecystokinin cholescintigraphy: victim of its own success? J. Nucl. Med. 1999, 40:2038-2042; Krishnamurthy S., et al., Gallbladder ejection fraction: A decade of progress and future promise. J. Nucl. Med. 1992, 32:542-544; Krishnamurthy GT., et al., Quantitative biliary dynamics: introduction of a new noninvasive scintigraphic technique. J. Nucl. Med. 1983;24:217 –223; Mesgarzadeh M., et al., Filling, post-cholecystokinin emptying and refilling of normal gallbladder: effects of two different doses of CCK on refilling: Concise Comm. J. Nucl. Med. 1983, 24:666-671; Krishnamurthy GT., et al., The gallbladder emptying response to sequential exogenous cholecystokinin, Nucl. Med. Com., 1984, 5 (1) pp 27-33; Krishnamurthy GT., et al., Detection, localization, and quantitation of degree of common bile duct obstruction by scintigraphy, J. Nucl. Med. 1985, 26:726-735; Fink-Bennet D., et al., Cholecystokinin cholescintigraphic findings in the cystic duct syndrome, J. Nucl. Med. 1985, 26:1123-1128; Fink-Bennet D., The role of cholecystogogues in the evaluation of biliary tract disorders. Nucl.Med. Ann. 1985, Lenny Freeman and Heidi Weissman, eds., New York, Raven Press, 1985, pp. 107-132; Newman P., et al., A simple technique for quantitation cholecystokinin-HIDA scanning. British J. of Radiology, vol. 56, pp. 500-502, 1983; Pickleman J., et al. The role of sincalide cholescintigraphy in the evaluation of patients with acalculous gallbladder disease. Archives of Surgery, vol. 120, 693-697; Ziessman, HA., et al., Calculation of a gallbladder ejection fraction: Advantage of continuous sincalide infusion over the three-minute infusion method. J. Nucl. Med. 1992, 33:537-41; Sitzmann, JV., et al., Cholecystokinin prevents parenteral nutrition induced biliary sludge in humans, Surg. Gynecol. Obstet. 170:25-31, 1990; Teitelbaum DH., et al., Treatment of parenteral nutrition-associated cholestasis with cholecystokinin-octapeptide. J. Pediatr. Surg. 30:1082, 1995.

After administration of the hepatobiliary imaging agent, the hepatobiliary system of the patient can be imaged using an appropriate detection device. When a Tc-99m IDA

(Iminodiacetic acid) analog , such as CHOLETEC®is used as an imaging agent, a gamma camera can be employed to scan the body of the patient for radioactivity. Imaging of the gallbladder allows for the non-invasive measurement and analysis of various biliary motor functions, including the gallbladder ejection fraction (GBEF). Measurement of GBEF is clinically valuable in the diagnosis and management of certain gallbladder-related disorders, including chronic acalclulous cholecystitis (CAC). In particular, low GBEF has been found to have a >90% positive predictive value for CAC. Other changes in biliary dynamics may be used in the diagnosis of a variety of biliary disorders.

Methods for determining GBEF scintigraphically are known in the art, and are described in, for example, the references cited above. Sincalide aids in the analysis of biliary function, including the measurement of GBEF, through its physiological effects on the gallbladder, e.g. it ability to induce gallbladder contraction and emptying. One technique for measuring GBEF is to administer sincalide slowly as a 1-3 minute infusion and to calculate GBEF at the end of about 20 minutes. Alternatively, sincalide may be infused rapidly as a bolus, or as a slower continuous infusion ranging from 15 to 60 minutes. By inducing certain biliary functions during hepatobiliary imaging, sincalide aids in the identification of anomalies in such functions, which may be indicative of certain hepatobiliary diseases.

Administration of sincalide formulations can be via IV or IM injections: For IV administration the dose can be administered as a bolus or slow injection over time optionally with the aid of an infusion pump. The dose for IV administration is typically 0.005 to 0.04 μg/kg (bolus injection) or 0.005μg/kg in a series of 4- three minute injections. A dose of 0.02-0.04 μg/kg IV over 2-3 minutes, but up to 1 hour is described in the art. Injection rates of 0.58 μg/kg/ hour can also be employed with the use of an infusion pump. Other regimens starting at 10 ng/kg/hr and increasing to 160 ng/kg/hr are also known in the art. Bolus injection is not recommended in every case, but injection of 0.02 to 0.04 μg/kg over 2-3 minutes even up to 15 min. can be used to avoid spasm of the cystic duct or GB.

Doses for IM administration are generally higher and range from 0.1 to 0.4 μg/kg. In one embodiment the 0.4 μg/kg IM dose is generally preferred resulting in the greatest GB response with the fewest side effects. Further details on administration are provided in, for example, Mesgarzadeh M., et al., Filling, post cholecystokinin emptying and refilling of normal gallbladder: effects of two different doses of CCK on refilling, J. Nucl. Med. 1983, 24:666-671; Ziessmann HA., et al., Calculation of a gallbladder ejection fraction: Advantage of continuous sincalide infusion over the three-minute infusion method. J. Nucl. 1992, 33:537-541; Pickleman J, et al., The role sincalide cholescintigraphy in the evaluation of patients with acalculous gallbladder disease. Archives of Surgery, vol. 120, 693-697; Krishnamurthy GT., et al., The gallbladder emptying response to sequential exogenous cholecystokinin, Nucl. Med. Com., 1984, 5 (1) pp 27-33; Krishnamurthy GT., et al., Quantitative biliary dymanics: introduction of a new noninvasive scintigraphic technique. J. Nucl. Med. 1983, 24:217 –223; Fink-Bennet D., The role of cholecystogogues in the evaluation of biliary tract disorders. Nucl.Med. Ann. 1985, Lenny Freeman and Heidi Weissman, eds., New York, Raven Press, 1985, pp. 107-132; Balon H.R., et al. Society of Nuclear Medicine procedure guideline for hepatobiliary scintigraphy.

The sincalide formulations of the invention are also useful for treating patients receiving total parenteral nutrition (TPN). TPN induces biliary sludge, the development of cholestasis, and the formation of gall stones and other gallbladder related complications. Indeed, TPN associated cholestatis (TPN-AC) can be a fatal in some instances. The clinical implications of TPN-AC include increased rates of sepsis, cirrhosis, declined lymphocyte function, obstructive jaundice, liver failure, and increased mortality. Although the mechanisms by which these disorders develop have not been definitely established, biliary stasis, the reduction in gallbladder emptying, bile flow, and bile acid secretion that accompanies TPN, has been implicated in the pathogenesis of TPN-AC and other TPN-associated complications. By promoting biliary contraction and emptying, the administration of sincalide to a TPN patient can help to treat and prevent diseases and other complications associated with prolonged TPN.

For TPN patients the dose of 0.05μg/kg is typically given IV over 10 minutes as a daily infusion. In infants, to treat high bilirubin levels the dose is 0.02μg/kg IV or IM twice or 3 times daily with doses increasing up to 0.32 μg/kg. CCK induces not only GB contraction but also increases intrahepatic bile flow. Information on the treatment of TPN-patients is provided in, for example, Sitzmann, JV., et al., Cholecystokinin prevents parenteral nutrition induced biliary sludge in humans, Surg. Gynecol. Obstet. Vol. 170:25-31, 1990; Moss RL., et al., New approaches to understanding the etiology and treatment of total parenteral nutrition-associated cholestasis, Surg. Gynecol. Obstet. Vol. 8:140-147, 1999; Teitelbaum DH., et al., Treatment of parenteral nutrition-associated cholestasis with cholecystokinin-octapeptide. J. Pediatr. Surg. 30:1082, 1995; Teitelbaum DH. Parenteral nutrition-associated cholestasis, Current Opinion in Pediatrics 1997, 9:270-275; Teitelbaum DH., et al., Parenteral nutrition-associated cholestasis. Seminars in Pediatric Surgery, Vol. 10, pp. 72-80.

The present invention is illustrated by the following examples, which are in no way intended to be limiting of the invention.

EXAMPLE 1

Effect of Buffering Agent and Formulation pH on Sincalide Formulations

Experiments were conducted to determine the effect of pH on the chemical stability of sincalide. Chemical instability, or degradation, may be caused by, for example, oxidation, reduction, deamidation, hydrolysis, imide formation, racemization, isomerization, and/or β-elimination. To examine the effect of pH on sincalide in phosphate buffer solution, solutions of sincalide (≈1.7 μg/mL) were prepared in 35 mM phosphate buffer and pH-adjusted with either dilute HCl or NaOH for final pH values ranging from 3.0 - 9.1. Using reverse-phase HPLC (RP-HPLC) with gradient elution and

UV detection at 215 nm, sincalide stability in solution was assessed by measuring the recovery of sincalide at 0, 6, and 24 hours after pH adjustment.

Results of the 24-hour study on the stability of sincalide in phosphate buffer over the pH range of 3.0 - 9.1 are summarized in Table 3 and also represented graphically in FIG. 5. By measure of the percentage recovery, sincalide was stable in 35 mM phosphate buffer solution at pH values ranging from 5.0 - 9.1 over a 24-hour period. At pH values < 5.0, sincalide degradation was evident even at the initial time point.

Table 3. Results of pH Study of Sincalide in 35 mM Phosphate Buffer

pH	n	Average % Sincalide Recovery		
		0 Hours	6 Hours	24 Hours
3.0	2	95.2 ± 0.4	93.4 ± 0.4	90.8 ± 1.2
4.0	2	93.0 ± 0.6	92.6 ± 1.6	85.5 ± 3.0
5.0	4	100.0 ± 2.7	99.8 ± 1.3	97.3 ± 1.8
5.5	2	100.7 ± 0.0	102.1 ± 0.3	101.6 ± 0.6
6.0	2	97.8 ± 0.4	99.8 ± 0.2	99.8 ± 1.0
6.5	2	98.8 ± 0.4	100.7 ± 0.3	99.6 ± 0.1
7.0	2	101.0 ± 0.0	101.0 ± 1.8	100.2 ± 1.2
7.5	2	101.0 ± 0.2	101.2 ± 0.8	100.4 ± 0.0
9.1	5	101.3 ± 2.3	101.1 ± 1.6	99.7 ± 0.9

Based on the results shown in Table 3, phosphate was selected as the buffering agent of choice due to a lack of interaction with sincalide and an ideal buffering capacity in the physiological pH range. Subsequently, experiments using phosphate in the formulation shown in Table 4 over the stable pH range established above were performed. Briefly, solutions of sincalide containing the following components (in the concentrations indicated in Table 4) were prepared: sincalide, D-mannitol, L-arginine, L-methionine, L-lysine, sodium metabisulfite, polysorbate 20, pentetic acid and dibasic potassium phosphate.

Table 4. Components of a Sincalide Formulation for Example 1

Component	Concentration (mg/vial)	Function
Sincalide	0.0050	Active
D-Mannitol	170.0	Bulking Agent/Tonicity Adjuster
L-Arginine Monohydrochloride	30.0	Stabilizer
L-Methionine	4.0	Stabilizer
L-Lysine Monohydrochloride	15.0	Stabilizer
Sodium Metabisulfite	0.040	Stabilizer
Polysorbate 20 (TWEEN®-20)	< 0.01	Surfactant
Pentetic Acid (DTPA)	2.0	Chelator
Dibasic Potassium Phosphate	9.0	Buffer

Solutions were pH-adjusted from 5.5 - 8.5 with dilute HCl or NaOH, and were evaluated for stability by measuring the sincalide recoveries at 0, 4, and 8 hours after pH adjustment, using RP-HPLC with gradient elution and UV detection at 215 nm, as described above. The results of an 8-hour study on the stability of sincalide in the above formulation over the pH range of 5.5 - 8.5 are summarized in Table 5 and also represented graphically in FIG. 6.

Table 5. Results of pH Study of a Preferred Lyophilized Sincalide Formulation of the Invention

pH	n	Average % Sincalide Recovery		
		0 Hours	4 Hours	8 Hours
5.5	2	99.7 ± 0.2	98.5 ± 0.1	98.1 ± 0.0
6.0	2	97.4 ± 0.5	98.0 ± 0.1	98.0 ± 0.2
7.0	2	98.4 ± 0.1	98.1 ± 0.1	97.5 ± 1.3
8.0	2	97.2 ± 0.6	95.4 ± 0.4	96.4 ± 0.2
8.5	1, 2, 2	99.2	98.0 ± 0.0	99.5 ± 0.9

No distinct pH-dependent related trends in initial sincalide recovery were observed over the pH range studied. Any fluctuation in sincalide recovery over time can be

attributed to normal assay variability and not degradation. Sincalide stability in this formulation is further supported by analyses of the chromatographic profiles for the presence of sincalide-related degradants which were consistent at 1.2-1.6% (impurity index) over the 8-hour study from pH 5.5 - 8.5. A bulk batch solution of sincalide formulation was prepared containing 25 mM phosphate, as a buffering agent, at a target pH value of 6.8 (range 6.7 - 6.9). Reconstitution of the lyophile with 5 mL of water is equivalent to 10 mM phosphate in the drug product. The data demonstrate solution stability over a physiologically compatible pH range and support a preferred pH of 6.0-8.0 for reconstituted sincalide.

EXAMPLE 2

Effect of Chelators on Sincalide Formulations

As shown in FIG. 1, the amino acid composition of sincalide includes two methionine (Met) residues which are designated as Met 3 and Met 6 in the structural sequence. Experiments were performed to determine whether these residues, as present in sincalide, were susceptible to oxidation by free metals. These experiments also examined the role of DTPA as a formulation excipient to chelate metals and thereby inhibit sincalide oxidation. FIGS. 2-4 show the three oxidized forms of sincalide containing either mono- or disulfoxides. As shown in Table 6, experimental formulations (without amino acids) at pH 6.5-7.0, with 1 mM DTPA (0.39 mg DTPA/mL) and without DTPA were prepared to evaluate potential oxidative effects due to the presence of metals.

Table 6. Sincalide Formulations Used in Example 2 (without Amino Acids)

Component	Concentration (mg/vial)	Bulk Concentration (mg/mL)
Sincalide	0.0050	0.0025
D-Mannitol	170.0	85.0
L-Arginine Monohydrochloride	0	0

L-Methionine	0	0
L-Lysine Monohydrochloride	0	0
Sodium Metabisulfite	0.040	0.02
Polysorbate 20	<0.01	2.5×10^{-6}
Pentetic Acid (DTPA)	(+) / (-)	1.0 / 0
Dibasic Potassium Phosphate	9.0	4.5

The experimental formulation (25 mL) solution with (+) and without (-) DTPA were individually spiked with nine metal ions, as summarized in Table 7.

Table 7. Evaluation of Metal Ions for Oxidative Effects on Sincalide

Metal	Volume (µL) of Metal Ion Standard	Metal Ion Concentration	1 mM DTPA (+) with / (-) without
Aluminum (Al^{3+})	100	1.48 mM 40 ppm	+ -
Chromium (Cr^{3+})	25	0.19 mM 10 ppm	+ -
Copper (Cu^{2+})	100	0.63 mM 40 ppm	+ -
Iron (Fe^{3+})	25	0.18 mM 10 ppm	+ -
Lead (Pb^{2+})	100	0.19 mM 40 ppm	+ -
Magnesium (Mg^{2+})	50	0.82 mM 20 ppm	+ -
Manganese (Mn^{2+})	25	0.18 mM 10 ppm	+ -
Nickel (Ni^{2+})	100	0.68 mM 40 ppm	+ -
Zinc (Zn^{2+})	100	0.61 mM 40 ppm	+ -

The metal-containing solutions were analyzed within 8 hours for sincalide and related oxidized forms by RP-HPLC with gradient elution and UV detection at 215 nm, as described above. FIG. 7 shows the effects of the nine metals in the presence and absence of DTPA on the formation of sulfoxides (Met 3 and Met 6). These data show

that, with the exception of Cr^{3+}, the amounts of sincalide Met 3 and Met 6 monosulfoxides increase in the presence of certain metals and in the absence of DTPA, while the presence of DPTA has an inhibitory effect on the formation of sincalide Met 3 and Met 6 monosulfoxides. Copper and manganese, in the absence of DTPA, have the greatest oxidative effect on the methionine residues of sincalide resulting in combined weight percentages of Met 3 and Met 6 monosulfoxides (vs sincalide) of 85.5 and 128.9, respectively. In addition to the presence of sincalide Met 3 and Met 6 monosulfoxides ($t_R \approx 14.8$ min. {doublet} and $t_R \approx 18$ min.), formation of sincalide disulfoxide ($t_R \approx 6.5$ min.) was also noted in the cases of copper and manganese, but not with the other metals.

Chromatograms of formulations spiked with copper or manganese (Figures 8-11) andwith or without DTPA also support this conclusion. The analyses of the chromatographic profiles indicate that levels of DTPA at 1 mM (0.39 mg DTPA/mL) protect sincalide from metal-catalyzed oxidation to sulfoxides. As trace metals often arise in formulations as a result of excipient impurities and/or stopper extractables, the results of the study support the use of pentetic acid (DTPA) as a formulation excipient to chelate trace levels of free metals, thus reducing the formation of sincalide methionine mono- and disulfoxides and inhibiting the degradation of sincalide in solution. Sincalide formulations were prepared containing 2 mg DTPA/vial, equivalent to 1 mM upon reconstitution with 5 mL.

EXAMPLE 3

Effect of Surfactants on Sincalide Formulations

During the preliminary developmental studies of a new formulation that consisted of bulking agent/tonicity adjuster, buffer, salt, chelator, and sincalide, it was observed by HPLC analysis that the recovery of the active pharmaceutical ingredient, sincalide, in the bulk solution was sensitive to standing open to air. For example, when using reversed-phase gradient elution HPLC with UV detection at 215 nm to monitor sincalide potency,

a substantial decrease of 50 - 60% in sincalide recovery was observed in unstoppered vials with a 2-mL fill of bulk solution either stirred or left standing open to air for 17 hours. Although to some extent, this sincalide decrease can be accounted for by an increase in the presence of sincalide mono- and disulfoxide degradants, these represent only a very minor percentage of the decreases noted. Thus the decrease in recovery is thought to be attributed to either adsorption/denaturing or air/liquid interface effects. To minimize sincalide degradation associated with surface adsorption, surfactants are added as formulation excipients in bulk and lyophilized formulations of sincalide.

Sincalide formulations consisting of a bulking agent/tonicity adjuster (D-mannitol), buffer (mono- and dibasic potassium phosphate), salt (sodium/potassium chloride) for tonicity, chelator (pentetic acid), and active ingredient (sincalide) were prepared using varying concentrations of the nonionic surfactant, polysorbate 80 (TWEEN® 80). Bulk solution and reconstituted lyophilized samples were either stoppered immediately or left unstoppered for 17 hours, and were assayed for sincalide recovery by reversed-phase gradient elution HPLC at 215 nm.

As shown in Table 8, the effect of TWEEN® 80 is more apparent in formulations that have been exposed to air. For bulk and reconstituted lyophilized formulations, the data show decreases in sincalide recovery of ≈ 50% and ≈ 20%, respectively, when compared to corresponding formulations containing a TWEEN® 80 concentration of 1 mg/mL. Low sincalide recoveries in closed bulk and reconstituted lyophilized formulations without TWEEN® 80 are also evident, but not nearly as substantial (4 - 8%) as the exposed formulations. These preliminary screening studies on the influence of TWEEN® 80 concentration indicate that < 1 mg/mL bulk may be optimal.

Table 8. Sincalide Recovery in Formulations With and Without TWEEN® 80

Formulation Description (mg/mL Bulk)	Test Condition	TWEEN® 80 Conc. (mg/mL)	Sincalide % Recovery
D-Mannitol (75.0), KH_2PO_4 (3.25), K_2HPO_4(1.0), NaCl (5.0), DTPA (1.0), Sincalide (0.0025), TWEEN® 80 (0; 0.1; 1.0)	Bulk; open (~17 h)	1.0	97.0
		0.0	47.0
	Bulk; closed	1.0	100.0
		0.0	96.0
	Lyophilized; open (~17 h)	1.0	91.3
		0.1	98.2
		0.01	98.3
		0.0	78.4
	Lyophilized; closed	1.0	90.2
		0.1	98.1
		0.01	97.8
		0.0	92.3

To compare the effects of two nonionic surfactants, sincalide formulations (75 mg/mL D-mannitol, 6.0 mg/mL KCl, 3.25 mg/mL KH_2PO_4, 1.0 mg/mL K_2HPO_4, 1.0 mg/mL DTPA, 0.0025 mg/mL sincalide (Bulk formulation)) were prepared using either TWEEN® 20 or TWEEN® 80 in varying amounts. The results of this experiment are presented in Table 9.

Table 9. Effect of Surfactants on Sincalide Recovery

Sincalide Formulation	TWEEN® Concentration (μg/mL Bulk)	Sincalide Recovery (%)
TWEEN® 80		
A	7.5	95.4
B	5.0	96.3
C	2.5	98.6
G	0	94.1
TWEEN® 20		
D	7.5	99.5
E	5.0	101.3
F	2.5	98.7
G	0	94.1

As shown in Table 9, the data indicate that the presence of trace levels (2.5 - 7.5 μg/mL) of either TWEEN® 80 or TWEEN® 20 has a beneficial effect on the recovery of sincalide, when compared to formulations without surfactant. However, the sincalide recoveries (98 - 102%) with formulations containing TWEEN® 20 are consistently higher than recoveries (95 - 98%) with TWEEN® 80, and thus TWEEN® 20 is a preferred surfactant.

An additional experiment was performed to confirm the effect of the concentration of TWEEN® 20 in terms of sincalide recovery in both air exposed and sealed bulk formulation. Sincalide recovery, determined for bulk formulation (75.0 mg/mL D-mannitol, 6.0 mg/mL KCl, 3.25 mg/mL KH_2PO_4, 1.0 mg/mL DTPA, 0.0025 mg/mL sincalide) containing varying trace levels of TWEEN® 20 stored in open or closed vials using reversed-phase gradient elution HPLC, is shown in Table 10.

Table 10. Effect of TWEEN® 20 Concentration on Recovery of Sincalide in Bulk Formulations

Sincalide Formulation	TWEEN® 20 Concentration (μg/mL Bulk)	Sincalide % Recovery	
		Open Vial	Closed Vial
D	7.5	100.7	100.8
E	5.0	100.0	100.4
F	2.5	99.0	98.2
G	0	89.8	96.1

As shown in Table 10, the bulk formulations containing TWEEN® 20 have improved sincalide recoveries over formulations with no TWEEN® 20 and the sincalide recoveries are independent of the TWEEN® 20 concentration range (2.5 - 7.5 μg/mL bulk) studied. In addition, the air sensitivity relative to sincalide recovery was eliminated, as both open and closed formulations containing TWEEN® 20 have equivalent sincalide recoveries. Although these data support the use of TWEEN® 20, it was noted that 2-mL filled vials containing a TWEEN® 20 concentration of 5 μg/mL show slight foaming in the reconstituted product upon vigorous stirring. To reduce foaming, a lower TWEEN® 20 concentration was evaluated.

As summarized in Table 11, an experiment was conducted on the lyophilized product comparing the recovery of the sincalide in the formulations with TWEEN® 20 (2.5 ng/mL) and without TWEEN® 20. In this Example and the subsequent Examples, mannitol refers to D-mannitol, methionine refers to L-methionine, arginine refers to L-arginine monohydrochloride, and lysine refers to L-lysine monohydrochloride.

Table 11. Sincalide Recovery in Reconstituted Formulations With and Without TWEEN® 20

Formulation Description (mg/mL Bulk)	Sincalide % Recovery	
	TWEEN® 20 Concentration	
	0 ng/mL	2.5 ng/mL
Mannitol (85.0), KH_2PO_4 (4.5), DTPA (1.0), Methionine (2.0), Lysine (7.5), Arginine (15.0), Sodium metabisulfite (0.02), Sincalide (0.0025)	94.8 (n = 5)	
		100.0 (n = 2)
		100.0 (n = 2)
		99.0 (n = 2)
Average	94.8	99.7
Variance	0.862	0.667
P(T<=t) two-tail	1.6×10^{-5}	

Reducing the amount of TWEEN® 20 to a minimal trace concentration (2.5 ng/mL) still produced a significant effect on the air/liquid interface and eliminated the foaming in the formulation. A statistical two-tail t-test performed on the results showed a significant difference ($P < 0.05$) between 2.5 ng/mL and no TWEEN® 20 in the formulation. Based on these data, the effectiveness of TWEEN® 20, polyoxyethylene sorbitan monolaurate, as a surfactant was established by enhancing the sincalide recovery and thus maintaining sincalide potency in the formulation. A preferred formulation of sincalide includes the nonionic surfactant TWEEN® 20 as a trace excipient at a

concentration of 2.5 ng/mL of bulk formulation equivalent to 1 ng/mL in the final product when reconstituted to 5 mL.

EXAMPLE 4

Effect of Antioxidants on Sincalide Formulations

An experiment was performed to evaluate the addition of antioxidants as stabilizing agents to prevent sincalide oxidation in formulations of sincalide (formulations for Example 4 contained 85 mg/mL mannitol, 0.005 mg/mL TWEEN® 20, 2.75 mg/mL KH_2PO_4, 1.0 mg/mL DTPA, 2.0 mg/mL methionine, 7.5 mg/mL lysine, 15 mg/mL arginine, 0.0025 mg/mL sincalide (Bulk formulation), except placebos which contained no sincalide.) The formation of sincalide methionine (Met 3 or Met 6) monosulfoxides, desulfated sincalide and unknown degradants was investigated. The effectiveness of sodium metabisulfite, ascorbic acid, cysteine, glutathione, sodium sulfate, benzalkonium chloride, and benzethonium chloride in inhibiting the degradation of sincalide in terms of their effect on sincalide recovery and sincalide-related impurities, was evaluated by HPLC.

The effect of various antioxidants on the stabilization of sincalide was evaluated on open and sealed sincalide formulations over 15 hours. The antioxidants were separately added at a concentration of 10 μg/mL to water-reconstituted lyophilized sincalide formulations containing all formulation ingredients except antioxidant. Spiked and unspiked solutions were pooled, subdivided, and either exposed to or protected from air over 15 hours. The sincalide and total sincalide-related impurities were monitored by reversed-phase HPLC with gradient elution and UV detection at 215 nm to compare the effectiveness of the antioxidants.

As shown in Table 12, the data at these concentrations indicate that benzalkonium chloride and benzethonium chloride had a significant destabilizing effect on sincalide, while ascorbic acid, cysteine, glutathione, and sodium sulfate were essentially equivalent

to the control formulation (no antioxidant). Of all the sincalide formulation/antioxidant combinations examined, the formulation with 10 μg sodium metabisulfite/mL showed the highest sincalide potency (98.3%) over 8 hours, and the lowest total sincalide-related impurities (1.79%) through 15 hours. Therefore, sodium metabisulfite is a preferred antioxidant for formulations of the invention.

Table 12. Effect of Various Antioxidants (10 μg/mL) on Sincalide Formulation Stability

Antioxidant (10 μg/mL)	% Sincalide						% Total Sincalide-Related Impurities					
	Sealed			Open			Sealed			Open		
	0 h	7 h	14 h	1 h	8 h	15 h	0 h	7 h	14 h	1 h	8 h	15 h
Control (None)	98.1	98.1	98.1	98.1	98.2	98.2	1.94	1.95	1.86	1.90	1.85	1.81
Sodium Metabisulfite	98.3	98.3	98.3	98.2	98.3	98.2	1.67	1.66	1.73	1.76	1.69	1.79
Ascorbic Acid	98.1	98.0	97.8	98.0	98.0	97.8	1.95	2.05	2.25	2.00	2.01	2.16
Cysteine	98.2	98.1	98.1	97.8	97.7	98.0	1.85	1.87	1.91	2.20	2.32	2.05
Glutathione	98.1	98.3	98.2	98.1	98.2	97.9	1.90	1.74	1.82	1.94	1.85	2.13
Sodium Sulfate	98.2	98.1	98.2	98.3	98.2	98.1	1.76	1.90	1.81	1.70	1.78	1.92
Benzalkonium Chloride	97.8	97.7	97.4	82.7	88.4	82.9	2.21	2.34	2.58	17.3	11.6	17.1
Benzethonium Chloride	97.9	98.0	98.0	92.1	88.0	92.6	2.13	1.98	1.96	7.93	12.0	7.36

To optimize the level of sodium metabisulfite in the formulation, lyophilized sincalide formulations were prepared containing four levels of sodium metabisulfite (0, 10, 30, and 60 μg/vial), as summarized in Table 13. Samples at each concentration were maintained under unstressed and stressed (65 °C, 64 hours) storage conditions, and were subsequently assayed by HPLC. The "% sincalide" was determined, and the "% (Met 6) monosulfoxide" (t_R ~19.7 min.) was monitored as an indication of sincalide oxidation. The data are presented in Table 14.

Table 13. Sincalide Lyophilized Formulations

Formulation No.	Formulation Description
1	complete formulation, no sodium metabisulfite
2	complete formulation, 10 μg sodium metabisulfite/vial
3	complete formulation, 30 μg sodium metabisulfite/vial
4	complete formulation, 60 μg sodium metabisulfite/vial
5	placebo(no sincalide), 40 μg sodium metabisulfite/vial
6	complete formulation, no sodium metabisulfite
7	complete formulation, 40 μg sodium metabisulfite/vial

Table 14. Effect of Sodium Metabisulfite Concentration on Sincalide Oxidation

Formulation	Sodium Metabisulfite Concentration (μg/vial)	% (Met 6) Monosulfoxide		% Sincalide	
		Unstressed	Stressed (65°C, 64 h)	Unstressed	Stressed (65°C, 64 h)
1	0	0.08	0.20	95.7	95.1
2	10	0.07	0.09	95.1	96.0
3	30	0.07	0.10	95.0	96.9
4	60	0.06	0.08	95.8	96.2

The addition of sodium metabisulfite up to 60 μg/vial improved sincalide recovery and inhibited the oxidation of sincalide to the (Met 6) monosulfoxide derivative under stressed conditions. Based on this data, as there was no apparent concentration relationship, 40 μg/vial sodium metabisulfite was selected as the preferred concentration for the final formulation, using 30 μg/vial and 60 μg/vial as lower and upper limits, respectively.

Another experiment was conducted under longer-term accelerated storage conditions utilizing a sincalide formulation with the optimized concentration (40 μg/vial) of sodium metabisulfite to confirm the protective effect on the degradation of sincalide. Sincalide lyophilized formulations with and without the antioxidant from the same batch were heat-stressed at 40°C and 60°C for 6 weeks. Also, formulations without sincalide from the same batch were heat-stressed at 40°C for 8 months. The results of the HPLC analyses for % sincalide and % total impurity are presented in Table 15.

Table 15. Effect of Sodium Metabisulfite on Heat Stress-Related Impurities

Formulation	Sodium Metabisulfite Concentration (μg/vial)	Storage Temp. (6 weeks)	% Sincalide	% Total Impurity
7	40	40°C	96.7	3.30
6	0	40°C	93.4	6.56
7	40	60°C	89.5	10.51
6	0	60°C	84.0	16.00

The results of this longer-term accelerated storage experiment further emphasized the need for the presence of the excipient sodium metabisulfite. Sincalide formulations with sodium metabisulfite (40 μg/vial) protected against sincalide heat stress-related degradant formation (3.30%), as compared without the antioxidant, which exhibited several elevated sincalide heat stress-related impurities (6.56%). These impurities were confirmed to be sincalide heat stress-related (t_R = 35 to 44 min.), as they were not present in chromatograms of formulations without sincalide. Sodium metabisulfite was chosen as a preferred antioxidant and stabilizing agent over ascorbic acid, cysteine, glutathione, sodium sulfate, benzalkonium chloride, and benzethonium chloride because it provided superior protection in inhibiting the oxidative and heat stress-related degradation of sincalide. A preferred concentration in the lyophilized formulation is 40 μg sodium metabisulfite/vial or 8 μg/mL in the reconstituted product.

EXAMPLE 5

Selection of Bulking Agent/Tonicity Adjuster

Due to the minute amount of the active pharmaceutical ingredient (API), sincalide (5 μg/vial), in the formulations of the invention, the use of a bulking agent was considered extremely beneficial for providing tonicity as well as for providing both structure and support for the API. Experiments were conducted for the selection and optimization of bulking agent in the sincalide formulations of the invention. Criteria for evaluation were: an efficient lyophilization cycle, a pharmaceutically elegant finished

product, enhanced product solubility and usefulness as an excipient for isotonicity in the reconstituted product. Various concentrations of lactose, lactose/sodium chloride and mannitol were considered, and experimental batches containing these excipients were evaluated in terms of cake appearance, osmolality, dissolution rate, and thermal analysis including freeze dry microscopy, and electrical resistance vs. temperature measurements.

Experimental Formulations

Batch A: ingredients: lactose 375 mg/vial, dibasic sodium phosphate 12.0 mg/vial, DTPA 2.0 mg/vial, monobasic sodium phosphate19.5 mg/vial , and 0.005 mg/vial sincalide.

Batch C_{1-3}: ingredients: mannitol 170 mg/vial, dibasic potassium phosphate 9.0 mg/vial, TWEEN® 20 <0.01 mg/vial, methionine 4.0 mg/vial, lysine 15.0 mg/vial, arginine 30.0 mg/vial, sodium metabisulfite 0.04 mg/vial, sincalide 0.005 mg/vial, and DTPA 2.0 mg/vial.

Batch D_1: ingredients: lactose 150 mg/vial, dibasic potassium phosphate 9.1 mg/vial, DTPA 2.0 mg/vial, monobasic sodium phosphate 9.8 mg/vial, and NaCl 21.0 mg/vial.

Batch E_1: ingredients: lactose 200 mg/vial, dibasic sodium phosphate 7.5 mg/vial, DTPA 2.0 mg/vial and NaCl 17 mg/vial.

Batch F_{1-2}: F_1: ingredients: mannitol 250 mg/vial, dibasic sodium phosphate 7.5 mg/vial, DTPA 2.0 mg/vial and sincalide 0 mg/vial; and

F_2: ingredients: mannitol 206 mg/vial, dibasic sodium phosphate 7.5 mg/vial, DTPA 2.0 mg/vial and sincalide 0.005 mg/vial.

Batch H_{1-2}: H_1: ingredients: mannitol 180 mg/vial, dibasic sodium phosphate 6.0 mg/vial, sincalide 0 mg/vial, NaCl 5 mg/vial and DTPA 2.0 mg/vial; and

H_2: ingredients: mannitol 150 mg/vial, dibasic potassium phosphate 4.5 mg/vial, sincalide 0.005 mg/vial, NaCl 10 mg/vial and DTPA 2.0 mg/vial.

Batch I $_{1-2}$: I_1: ingredients: mannitol 140 mg/vial, dibasic potassium phosphate 5.5 mg/vial, TWEEN® 20 0.01 mg/vial, methionine 4.0 mg/vial, lysine 60.0 mg/ vial, sincalide 0.005 mg/vial and DTPA 2.0 mg/vial; and

I_2: ingredients: mannitol 170 mg/vial, dibasic potassium phosphate 5.5 mg/vial, TWEEN® 20 0.01 mg/vial, methionine 4.0 mg/vial, lysine 30.0 mg/vial, sincalide 0.005 mg/vial and DTPA 2.0 mg/vial.

Batch J: ingredients: mannitol 170 mg/vial, dibasic potassium phosphate 8.5 mg/vial, TWEEN® 20 0.01 mg/vial, methionine 4.0 mg/vial, lysine 15.0 mg/vial, arginine 30.0 mg/vial, Na metabisulfite 0.04 mg/vial, sincalide and DTPA 2.0 mg/vial.

Methods:

1. Appearance: Visual assessment of the freeze-dried plug.
2. Osmolality: Determined by vapor pressure osmometry.
3. Dissolution: Dissolution time measured by visual inspection under an inspection light upon reconstitution with 5 mL of water.
4. Thermal Analysis:
   a. Electrical resistance vs. temperature measurements: Electrical resistance measured using a proprietary resistance instrument, temperature measured using a 32-gauge type T thermocouple.
   b. Freeze drying microscopy: Performed using a freeze dry microscope an Infinivar microscope and color camera.

In the initial investigations lactose was used as a bulking agent/tonicity adjuster. The formulation as listed in table 16 is based on a 3-mL fill volume with a high concentration of lactose to achieve isotonicity in the reconstituted product. The osmolality for this formulation upon reconstitution with 5 mL of water was ~ 300 mOsm/kg.

Table 16. Lactose Containing Sincalide Formulation (Batch A)

Raw Materials	Function	Concentration (mg/vial)
Lactose	Bulking Agent/ Tonicity Adjuster	375
Dibasic Sodium Phosphate	Buffer	12.0
Monobasic Sodium Phosphate	Buffer	19.5
Pentetic Acid	Chelator	2.0
Sincalide	Active	0.005

This experimental formulation, Batch A, with a lyophilization cycle of 130 hours (≈ 5.4 days) showed evidence of meltback in the lyophilized cakes and had reconstitution dissolution times of ≥ 9 minutes. The high number of vials with poor cake formation and the long freeze dry cycle required were attributed to the high concentration of lactose (125 mg/mL) in the bulk formulation relative to its solubility and the high fill volume (3-mL) in a small vial.

Studies were undertaken to reduce cycle time and improve product appearance/solubility by modifying the initial lactose formulation with the use of an additional excipient, sodium chloride, thereby reducing lactose concentration and the fill volume from 3 to 2-mL.

Table 17. Lactose/NaCl Containing Sincalide Formulations (Batches D_1 and E_{1-2})

Raw Materials	Function	Concentration (mg/vial)
Lactose	Bulking Agent/ Tonicity Adjuster	150 - 200
Dibasic Sodium Phosphate	Buffer	12.0
Monobasic Sodium Phosphate	Buffer	19.5
Pentetic Acid	Chelator	2.0
Sodium Chloride	Tonicity Adjuster	17 - 21
Sincalide	Active	0.005

Use of NaCl contributed to the isotonicity of the product with osmolality values in the range of 240 to 270 mOsm/kg, while permitting a reduction in the concentration of lactose. Varying the amounts of lactose, sodium chloride and sodium phosphate decreased the lyophilization cycle from 130 hours to 96 hours, but did not consistently improve the appearance of the freeze-dried cake.

Thermal analysis of two experimental formulations with varying lactose/sodium chloride ratios (Table 18) confirm that the relatively long lyophilzation cycles for these formulations were due to low primary drying temperatures in the range of -38°C to -42°C, resulting in slow sublimation rates at these temperatures. In addition to long lyophilization cycles, the low primary drying temperatures lead to increased vial-to-vial variation and an increased risk of poor plug appearance with associated solubility issues.

Table 18. Thermal Analysis of Experimental Lactose/NaCl Formulations

Batch	Lactose/NaCl Concentration (mg/vial)	Freezing Temp. Range (°C)		Primary Drying Temp. Range (°C)	
		High	Low	High	Low
D_1	150/21	-32	-39	-39	-42
E_1	200/17	-15	-35	-36	-38

Mannitol, a common excipient for freeze-dried pharmaceuticals, was selected next for evaluation as bulking agent because of the high melting temperature of the mannitol/ice eutectic mixture (about -1.5°C) and its tendency to crystallize from frozen aqueous solutions. Ideally, this leads to shorter primary and secondary drying times, promoting an efficient freeze-drying cycle and a physically stable, pharmaceutically elegant freeze-dried solid. Several bench-scale batches were prepared, replacing lactose with D-mannitol while maintaining isotonicity with a 2 mL fill volume, to evaluate the parameters of cycle time and primary drying temperature and the solubility of the solid cake. The freeze dry cycle parameters along with lyophilized product reconstitution times with a 5 mL reconstitution volume are shown in Table 19.

Table 19. Effect of Formulation Bulking Agent/Tonicity Adjuster on Lyophilization Cycle Optimization and Reconstitution/Dissolution Time

Batch	Formulation Description (mg/vial)	Bulking Agent (mg/vial)	Osmolality (mOsm/kg)	Freeze Dry Cycle Parameters	Dissolution Time (sec)
F_1	Na_2HPO_4 (7.5), DTPA (2.0)	Mannitol (250)	280	Total Cycle 85 hr Primary drying @ -34°C	12 – 48 (n = 10)
F_2	Na_2HPO_4 (7.5), DTPA (2.0)	Mannitol (206)	240	Total Cycle 69 hr Primary drying @ -25°C	22 – 71 (n = 30)

Lyo-cycle time was reduced from >130 hours for lactose formulations, to ~ 69 hours for the mannitol formulation, Batch F_2. The cakes from both formulations, F_1 and F_2 dissolved in 5 mL of water in approximately the same time range of < 1 minute. Increasing the primary drying temperature from ~ -34°C to -25°C Batch F_1 vs. Batch F_2 had the desired effect of reducing the overall cycle time from 85 to 69 hours.

Additional studies were conducted to optimize the mannitol concentration and lyo-cycle time for a 2-mL fill volume. These studies were carried out concurrently with formulation development studies to adjust the osmolality to ~ 250 mOsm/Kg after reconstitution and to stabilize the peptide by addition of other excipients to the formulation (Table 20).

Table 20. The Effect of Mannitol Concentration on Appearance, Solubility and Freeze Dry Cycle of Sincalide Formulations

Batch	Formulation Description (mg/vial)	Bulking Agent (mg/vial)	Osmolality (mOsm/kg)	Freeze Dry Cycle Parameters	Moisture Content (%)	Appearance/ Dissolution Time (sec)
H_1	Na_2HPO_4 (6.0), DTPA (2.0), Sincalide (0)	Mannitol (180) NaCl (5.0)	250	Total Cycle: 36 hr 27 hr primary @ -8°C	ND	Solid cake/ 22 – 66 (n = 30)
H_2	K_2HPO_4 (4.5), DTPA (2.0), Sincalide (0.005)	Mannitol (150) NaCl (10.0)	240	Total Cycle: 30 hr 23 hr primary @ -10°C	1	Solid cake/ 11 – 31 (n = 30)
I_1	TWEEN® (0.01), K_2HPO_4 (5.5), Methionine (4.0), Lysine (60.0), DTPA (2.0), Sincalide (0.005)	Mannitol (140)	250	Total Cycle: 59 hr 50 hr primary @ -22°C	1	Solid cake/ 21 – 69 (n = 5)
I_2	TWEEN® (0.01), K_2HPO_4 (5.5), Methionine (4.0), Lysine (30.0), DTPA (2.0), Sincalide (0.005)	Mannitol (170)	250	Total Cycle: 33 hr 26 hr primary @ -12°C	1	Solid cake/ 8 – 15 (n = 5)

ND=Not Determined

These results demonstrate that an increase in primary drying temperature from ~ -25°C to the -8 to -12°C range significantly reduced cycle times from 69 to 30 hours and produced solid dry cakes that reconstitute within 1 minute.

Additional optimization studies designed to enhance the long term stability of sincalide resulted in a preferred sincalide formulation of 170 mg of D-mannitol/vial with the additional excipients (in mg/vial): TWEEN® 20 (0.01), K_2HPO_4 (8.5), methionine (4.0), lysine (15.0), arginine (30.0), DTPA (2.0), and sodium metabisulfite (0.04). The osmolality of this optimized formulation was approximately 300 mOsm/kg when reconstituted with 5 mL of water. Thermal analysis of this formulation using freeze-dry microscopy and electrical resistance vs. temperature measurements, indicated an upper limit for product primary drying temperature of -13°C to -15°C to achieve acceptable product quality.

To confirm all findings, three scale-up pilot batches, C_{1-3}, of a preferred sincalide formulation , in a fill volume of 2 mL/vial, were prepared and freeze dried in full-scale production driers to prove process transferability from development equipment to production equipment. The drying cycle for these batches incorporated a primary drying temperature of -12°C ± 3°C and an overall cycle time of 53-61 hours (Table 21).

Table 21. Operating Parameters and Final Product Performance of Scale-up Pilot Batches Prepared with Mannitol as a Bulking Agent

Batch	Lyophilization		Osmolality (mOsm/kg)	Plug Appearance	Moisture Content %)	Dissolution Time (sec)
	Primary Temp (°C)	Total Cycle Time (Hrs)				
C_1	-12	58	300	Solid cake	1	10
C_2	-12	53	300	Solid cake	1	10
C_3	-12	61	300	Solid cake	1	10

The data from these studies support the selection of mannitol as a particularly preferred bulking agent, preferably in an amount of about 170 mg/vial. Using this concentration, the freeze dry cycle is 53-61 hours when filled as a 2-mL fill. The finished product is a pharmaceutically elegant, solid white cake, which is reconstituted within one minute using 5 mL of water, resulting in a solution with an osmolality of ~ 300 mOsm/Kg.

EXAMPLE 6

Effect of Amino Acids on Sincalide Formulations

During formulation studies it was observed that both exposure to air and lyophilization were areas of concern for scale-up manufacturing due to reduced potency of sincalide in the formulation. The reduced potency was a result of surface adsorption/denaturation resulting from exposure of sincalide to air, and yielding degradants via oxidation. Exposure of sincalide formulations to thermal stress during lyophilization also resulted in degradation and reduced recovery of sincalide.

Experiments were conducted to evaluate several amino acids as potential stabilizers of sincalide, including the non-polar (hydrophobic) methionine residue, aspartic acid and glutamic acid, the polar glycine and cysteine residues, and the basic lysine and arginine amino acids.

Except as otherwise indicated, the formulations used in this example for testing the efficacy of various amino acids contained the following ingredients (bulk): 75.0 mg/mL mannitol; 3.25 mg/mL KH_2PO_4; 1.0 mg/mL K_2HPO_4; 1.0 mg/mL pentetic acid (DTPA); 5.0 mg/mL NaCl; and the active peptide, sincalide (0.0025 mg/mL). Initially, the non-polar amino acid L-methionine was evaluated for the reformulation since methionine residues can act as endogenous antioxidants, or as scavengers by reacting with hydroxyl free radicals and other reactive oxygen species. Thus, methionine could improve the processing stability of sincalide formulations by providing a protectant or antioxidant effect for sincalide and being preferentially oxidized. Table 22 below summarizes the results obtained during exposure of experimental formulations to air when various amounts of L-methionine were added to a formulation containing mannitol, sodium chloride, potassium phosphate, and pentetic acid. For these experiments, liquid formulations in open and closed vials were used to simulate processing of the product. For formulation in open vials, the recovery of sincalide was improved approximately 60% and the concentration of sincalide-related impurities decreased as the level of methionine was increased from 0.0 to 2.0 mg/mL in the bulk formulation.

Table 22. Evaluation of Methionine as a Processing Stabilizer for Bulk Formulations - Open vs Closed Vials.

L-Methionine (mg/mL Bulk)	Sincalide Recovery (%)		Related Impurities (%)	
	Open	Closed	Open	Closed
2.0	75.5	95.7	15.0	0.7
0.50	64.7	94.8	19.3	0.8
0.025	35.7	93.9	35.9	1.0
0.00	13.9	95.7	52.7	1.3

For comparison to the non-polar amino acid methionine as a potential processing stabilizer, polar amino acids such as glycine and cysteine were also evaluated. Formulations containing these amino acids were exposed to air in open vials and compared to product in closed vials. The efficacy of these amino acids, in terms of sincalide recovery and sincalide-related impurities, was compared to the improvements previously observed for the liquid formulation in the presence of methionine. Table 23 presents the sincalide recoveries for experimental formulations containing variable concentrations of methionine, cysteine or glycine.

Table 23. Comparison of Methionine, Glycine and Cysteine as Processing Stabilizers for Bulk Formulations - Open vs. Closed Vials

Amino Acid (mg/mL Bulk)		Sincalide Recovery (%)		Related Impurities (%)	
		Open	Closed	Open	Closed
L-Cysteine	2.0	50.0	96.0	31.0	1.0
L-Methionine	2.5	82.4	97.4	10.5	0.7
L-Methionine	2.0	89.9	97.7	6.4	0.7
None	0	37.0	96.0	35.0	1.6
L-Glycine	2.5	31.9	93.2	44.9	1.6
L-Glycine	2.0	22.3	92.5	51.1	1.1

Results demonstrated that addition of either cysteine or glycine to a bulk formulation containing mannitol, potassium phosphate, sodium chloride and pentetic acid did not show a significant effect in either reduced levels of sincalide impurities or improved recovery of sincalide when formulations were exposed to air in open vials.

Lysine, a basic amino acid, was the next amino acid evaluated for use in sincalide formulations of the invention. As shown in Table 24, experimental formulations (70-85 mg/mL mannitol, 0.005 mg/mL TWEEN® 20, 2.75 mg/mL KH_2PO_4, 1.0 mg/mL DTPA,

2.0 mg/mL methionine, 0.0025 mg/mL sincalide) were prepared to contain varying concentrations of lysine and evaluated for sincalide recovery.

Table 24. Evaluation of Lysine as a Stabilizer in Sincalide Reconstituted Formulations

DL-Lysine (mg/mL Bulk)	Sincalide Recovery (%)					
	1 Week		3 Weeks		5 Weeks	
	5°C	40°C	5°C	40°C	5°C	40°C
0.0	99.6	84.3	95.5	51.2	NA	25.4
5.0	98.1	95.4	93.6	98.4		92.0
15.0	97.3	97.0	94.3	99.4		93.2
30.0	96.6	95.0	95.5	97.2		89.7

NA = Not Applicable

After accelerated storage, lyophilized formulations containing lysine resulted in significantly improved recoveries of sincalide compared to a lyophilized control formulation without lysine. Formulations containing lysine resulted in 50% and 75% improvements in sincalide recovery after 3 and 5 weeks storage at 40°C, respectively, demonstrating that lysine contributed to the stability of lyophilized formulations when subjected to thermal stress.

The improved sincalide recoveries observed in the presence of methionine and lysine suggested that other amino acids might also be suitable as bulk additives in the reformulation. Therefore, formulation studies continued with the evaluation of two acidic amino acids, aspartic acid and glutamic acid. Table 25 presents sincalide recoveries for experimental formulations (85.0 mg/mL mannitol, 0.005 mg/mL TWEEN® 20, 2.75 mg/mL KH_2PO_4, 1.0 mg/mL DTPA, 2.0 mg/mL methionine, 0.0025 mg/mL sincalide) containing the following amounts of either lysine, aspartic acid or glutamic acid.

Table 25 Comparison of Lysine, Aspartic Acid and Glutamic Acid as Stabilizers in Sincalide Reconstituted Formulations

Formulation		Amino Acid		Sincalide Recovery (%)		
Process Conditions	ID	(mg/mL)		0 Days	10 Days	30 Days
Liquid Bulk Stored 5°C	A	DL-Lysine HCl	15.0	99.9	98.4	NA
	B	L-Aspartic Acid	11.0	98.2	96.3	
	C	L-Glutamic Acid	12.0	97.3	96.3	
Lyophilized Cake Stressed 40°C	A	DL-Lysine HCl	15.0	NA	98.2	99.1
	B	L-Aspartic Acid	11.0		94.6	92.8
	C	L-Glutamic Acid	12.0		95.5	95.7
	E	Control	0.0		81.7	53.8

NA = Not Applicable

The results demonstrated that with increasing storage time at 40°C, lysine consistently provided better protection than either aspartic acid or glutamic acid. The results obtained for lysine also suggested that arginine, another basic amino acid, or potentially some combination of lysine and arginine, might further enhance protection during lyophilization and thermal stress. Experimental formulations (85.0 mg/mL mannitol, 0.005 mg/mL TWEEN® 20, 2.75 mg/mL KH_2PO_4, 1.0 mg/mL DTPA, 2.0 mg/mL methionine, 0.0025 mg/mL sincalide) were prepared to contain varying concentrations of lysine, arginine, or a combination of lysine and arginine and evaluated for sincalide recovery (Table 26).

Table 26. Evaluation of Lysine and Arginine as Stabilizers in Sincalide Reconstituted Formulations

Amino Acid (mg/mL Bulk)		Sincalide Recovery (%)	
		64 hrs. @ 65°C	1 week @ 40°C
DL-Lysine	15.0	88.4	ND
L-Arginine	17.5	93.0	
DL-Lysine	7.50	99.8	
L-Arginine	8.75		
DL-Lysine	7.5	93.8	96.4
L-Arginine	17.5		
DL-Lysine	5.0	91.2	ND
L-Arginine	11.7		
DL-Lysine	7.5	95.1	ND
L-Arginine	15.0		
N/A (control)	0.0	43.3	ND

NA = Not Applicable, ND = Not Determined

Results confirmed that after lyophilization and stressing for 64 hours @ 65°C, approximately 50-70% improvement in sincalide recovery was observed for formulations containing lysine, arginine, or a combination of the two. Formulations containing both lysine and arginine exhibited the highest sincalide recovery values, indicating that the combination of these two amino acids provided a particularly stabilizing effect under heat-stressed storage conditions. The mid-point combination of 7.5 mg/mL of lysine and 15.0 mg/mL of arginine afforded suitable protection for the lyophilized and heat-stressed product, resulting in sincalide recoveries of >95%.

Methionine, lysine and arginine are preferred over polar amino acids such as glycine and cysteine and acidic amino acids such as aspartic acid and glutamic acid for use as stabilizers in the sincalide formulations of the invention. Methionine improved the

processing stability of the formulation, resulting in improved recovery of sincalide, and the combination of lysine and arginine contributed to the stability of the product during lyophilization and heat-stressing, also resulting in improved recovery of sincalide. Preferred concentrations in lyophilized formulations of the invention are: methionine (4.0 mg/vial), lysine (15.0 mg/vial) and arginine (30.0 mg/vial).

EXAMPLE 7

Reconstituted Shelf-life Studies

A. In-Vial Post-Reconstitution Stability

Experiments were performed to determine the post-reconstitution stability of sincalide in terms of appearance, solubility, particulate matter, color, pH, sincalide assay, desulfated sincalide assay and other sincalide-related impurities through 8 hours at ambient temperature. Lyophilized vials from three 105-L scale-up pilot batches of sincalide formulations were reconstituted with 5.0 mL of purified water.

Testing was conducted at 0, 2, 4, 6, and 8 hours post-reconstitution for appearance, solubility, particulate matter, color, and pH. Testing was conducted on duplicate vials at 0, 4, and 8 hours post-reconstitution for sincalide assay, desulfated sincalide assay and other sincalide-related impurities using reversed-phase HPLC with gradient elution and UV detection at 215 nm.

The test results for appearance, solubility, particulate matter, color, and pH performed at 0, 2, 4, 6, and 8 hours post-reconstitution for the three sincalide formulation preparations are shown in Table 27.

Table 27. Post Reconstitution Test Results

Preparation	Time (hr)	Appearance	Solubility (sec.)	Particulate Matter	Color	pH	
						meter 1	meter 2
A	0	Clear	20	Complies	Colorless	7.2	7.0
	2	Clear	20	Complies	Colorless	7.1	7.0
	4	Clear	20	Complies	Colorless	7.2	7.0
	6	Clear	20	Complies	Colorless	7.2	7.0
	8	Clear	20	Complies	Colorless	7.2	7.0
B	0	Clear	20	Complies	Colorless	7.2	7.0
	2	Clear	20	Complies	Colorless	7.1	7.0
	4	Clear	20	Complies	Colorless	7.2	7.0
	6	Clear	20	Complies	Colorless	7.1	7.0
	8	Clear	20	Complies	Colorless	7.1	7.0
C	0	Clear	20	Complies	Colorless	7.1	7.0
	2	Clear	20	Complies	Colorless	7.1	7.0
	4	Clear	20	Complies	Colorless	7.1	7.0
	6	Clear	20	Complies	Colorless	7.1	7.0
	8	Clear	20	Complies	Colorless	7.1	7.0

For the three preparations examined (referred to herein as preparations A, B, and C), no changes were observed in the parameters tested and all results were within specifications through the 8-hour testing period (85 mg/mL mannitol; 2.5 x 10^{-6} mg/mLTWEEN® 20; 4.5 mg/mL KH_2PO_4; 1.0 mg/mL DTPA, 0.02 mg/mL sodium metabisulfite, 2.0 mg/mL methionine, 7.5 mg/mL lysine, 15.0 mg/mL arginine, 0.0025 mg/mL sincalide (Bulk formulation). The HPLC test results for sincalide assay, desulfated sincalide assay and other sincalide-related impurities performed at 0, 4, and 8 hours post-reconstitution for the three formulation preparations are shown in Table 28.

Table 28. Post Reconstitution HPLC Test Results

Preparation	Time (h)	Sincalide (μg/vial)	Desulfated Sincalide (w/w % sincalide)	Sincalide Related Impurities (% Impurity Index)
A	0	4.99, 4.98	0.32, 0.33	1.41, 1.32
	4	4.99, 4.97	0.32, 0.36	1.40, 1.35
	8	4.97, 4.97	0.35, 0.39	1.40, 1.34
B	0	5.04, 5.04	0.28, 0.27	1.29, 1.37
	4	5.04, 5.03	0.30, 0.29	1.30, 1.39
	8	5.03, 5.01	0.31, 0.31	1.44, 1.41
C	0	4.97, 4.94	0.36, 0.36	1.48, 1.33
	4	4.97, 4.94	0.39, 0.37	1.41, 1.37
	8	4.97, 4.92	0.44, 0.44	1.46, 1.41

All results were within specifications. The sincalide potency was unchanged over time. The desulfated sincalide and other sincalide-related impurities show only relatively minor increases which are insignificant with respect to their individual specifications of 2% and 5%, respectively. The study shows that the initial test values of reconstituted sincalide formulations are representative of results obtained throughout the 8-hour shelf life of reconstituted product. The data provided demonstrate the post-reconstitution stability of the formulation and support a post-reconstitution shelf-life of 8 hours under ambient conditions.

B. Post-Reconstitution Dilution Study

An experiment was performed to determine the stability of sincalide formulations of the present invention that have been reconstituted and diluted.

Duplicate vials from three 105-L batches of sincalide formulations of the invention were reconstituted with 5 mL water. Vial contents were quantitatively transferred (using Sodium Chloride Injection USP to rinse) to 100-mL volumetric flasks and up to 8.4 mL of the formulations were diluted to volume (100mL) with Sodium Chloride Injection USP. Sincalide potency, pH and visual appearance were tested 1-hour post preparation. The results of the testing are presented in Table 29.

Table 29. Results for Sincalide Formulations to 100 mL With 0.9% Saline at 1 Hour Post-Reconstitution

Preparation	Sample No.	Sincalide Potency (µg/vial)	pH	Appearance	Color	Particulate Matter
A	1	4.8	6.9	Clear	Colorless	Free of particles
	2	5.0	6.9	Clear	Colorless	Free of particles
B	1	5.2	6.9	Clear	Colorless	Free of particles
	2	4.9	6.8	Clear	Colorless	Free of particles
C	1	4.9	6.9	Clear	Colorless	Free of particles
	2	4.9	6.8	Clear	Colorless	Free of particles
Mean		5.0	6.9			
Std. Dev.		0.1	0.1			
Confidence Interval (p=0.95 and 5 deg. Freedom)		4.8 - 5.1	6.8 - 6.9			
CV (%)		2.8	0.8			

All sincalide potency, pH and appearance results for diluted samples (reconstituted vial contents further diluted to 100 mL) measured 1-hour post reconstitution were within the product specifications for the reconstituted product (vial reconstituted with 5 mL water).

EXAMPLE 8

Sincalide Specific Assay using HPLC

An HPLC method was developed and validated for the determination of sincalide potency, quantitation of desulfated sincalide impurity and determination of a sincalide-related impurity index in KINEVAC®. The method is suitable for determining the reconstituted stability of KINEVAC® when reconstituted as per the product package insert. The reversed phase method employs a C_{18} (5 µm, 300 Å) column, stepwise gradient elution with mobile phase components consisting of 0.15% trifluoroacetic acid in water (solvent A) and 0.125% trifluoroacetic acid in acetonitrile (solvent B), and UV detection at 215 nm.

FIG. 12 shows representative full-scale and expanded-scale chromatograms of a lyophilized reformulation of KINEVAC® upon reconstitution with 5 mL water, resulting in a sincalide concentration of 1 μg/mL.

Other Embodiments

Although the present invention has been described with reference to preferred embodiments, one skilled in the art can easily ascertain its essential characteristics, and without departing from the spirit and scope thereof can make various changes and, modifications of the invention to adapt it to various usages and conditions. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are encompassed by the scope of the present invention.

All publications, patents, and applications mentioned in this specification are herein incorporated by reference.

We claim:

SEQ #

Claims

1. A stabilized, physiologically acceptable formulation of sincalide comprising:
(a) an effective amount of sincalide,
(b) at least one stabilizer,
(c) a surfactant/solubilizer
(d) a chelator,
(e) a bulking agent/tonicity adjuster, and
(f) a buffer.

2. The formulation of claim 1 having a pH from 6.0 to 8.0.

3. The formulation of claim 1, wherein said buffer is selected from the group consisting of phosphoric acid, phosphate, citric acid, citrate, sulfosalicylate, acetic acid, acetate, methyl boronic acid, boronate, disodium succinate hexahydrate, one or more amino acids, lactic acid, lactate, maleic acid, maleate, potassium chloride, benzoic acid, sodium benzoate, carbonic acid, carbonate, bicarbonate, boric acid, borate, sodium chloride, succinic acid, succinate, tartaric acid, tartrate, tris-(hydroxymethyl)aminomethane, and biological buffers.

4. The formulation of claim 1, wherein said buffer is a phosphate buffer.

5. The formulation of claim 4, wherein said buffer is dibasic potassium phosphate.

6. The formulation of claim 1, wherein said surfactant/solubilizer is selected from the group consisting of anionic surfactants, pluronics, poloxamers, SDS, Triton-100, polysorbates, propylene glycol, PEG and similar compounds, Brij58 9poly(oxyethylene)20 cetyl ether, cremophor EL, cetyl trimethylammonium

bromide (CTAB), dimethylacetamide (DMA), NP 40 (Nonidet P-40), and N-methyl-2-pyrrolidone (Pharmasolve), and amino acids.

7. The formulation of claim 1, wherein said surfactant is a nonionic surfactant.

8. The formulation of claim 7, wherein said nonionic surfactant is a polysorbate.

9. The formulation of claim 7, wherein said nonionic surfactant is polysorbate 20 or polysorbate 80.

10. The formulation of claim 1, wherein said stabilizer is selected from the group consisting of antioxidants and amino acids.

11. The formulation of claim 10, wherein said stabilizer is an antioxidant.

12. The formulation of claim 11, wherein said stabilizer is sodium metabisulfite.

13. The formulation of claim 1, wherein said formulation comprises a plurality of stabilizers.

14. The formulation of claim 13, wherein said stabilizers comprise L-arginine monohydrochloride, L-methionine, L-lysine monohydrochloride, and sodium metabisulfite.

15. The formulation of claim 1, wherein said bulking agent/tonicity adjuster is selected from the group consisting of mannitol, amino acids, lactose, potassium chloride, sodium chloride, maltose, sucrose, PEG's, trehalose, raffinose, dextrose, cyclodextrins, dextran, galacturonic acid, Ficoll, and polyvinylpyrrolidone (PVP).

16. The formulation of claim 15, wherein said bulking agent/tonicity adjuster is D-mannitol.

17. The formulation of claim 1, wherein said chelator is pentetic acid (DTPA).

18. The formulation of claim 17, wherein said chelator is pentetic acid, said surfactant is polysorbate 20, said buffer is dibasic potassium phosphate, and said bulking agent/tonicity adjuster is D-mannitol.

19. The formulation of claim 1, wherein said formulation is suitable for parenteral administration.

20. A stabilized, physiologically acceptable formulation of sincalide comprising:
(a) about 0.0008-0.0012 mg/mL sincalide,
(b) about 20 to 50 mg/mL mannitol,
(c) about 2 to 7 mg/mL arginine,
(d) about 0.2 to 1 mg/mL methionine,
(e) about 2 to 30 mg/mL lysine,
(f) about 0.002 to 0.012 mg/mL sodium metabisulfite,
(g) about 0.000001 to 0.003 mg/mL polysorbate 20,
(h) about 0.1 to 3 mg/mL pentetic acid (DTPA), and
(i) about 1.1 to 1.8 mg/mL dibasic potassium phosphate.

21. A method for making a stabilized formulation of sincalide, said method comprising the step of mixing: (a) at least one stabilizer, (b) a surfactant/solubilizer, (c) a chelator, (d) a bulking agent/tonicity adjuster, (e) a buffer (f) an aqueous solution, and (g) sincalide.

22. The method of claim 21, wherein said formulation has a pH from 6.0 to 8.0.

23. The method of claim ~~21,~~ wherein said buffer is selected from the group consisting of phosphoric acid, phosphate, citric acid, citrate, sulfosalicylate, acetic acid, acetate, methyl boronic acid, boronate, disodium succinate hexahydrate, one or more amino acids, lactic acid, lactate, maleic acid, maleate, potassium chloride, benzoic acid, sodium benzoate, carbonic acid, carbonate, bicarbonate, boric acid, borate, sodium chloride, succinic acid, succinate, tartaric acid, tartrate, tris-(hydroxymethyl)aminomethane, and biological buffers.

24. The method of claim 21, wherein said buffer is a phosphate buffer.

25. The method of claim ~~24,~~ wherein said buffer is dibasic potassium phosphate.

26. The method of claim 21, wherein said surfactant/solubilizer is selected from the group consisting of anionic surfactants, pluronics, poloxamers, SDS, Triton-100, polysorbates, propylene glycol, PEG and similar compounds, Brij58 9poly(oxyethylene)20 cetyl ether, cremophor EL, cetyl trimethylammonium bromide (CTAB), dimethylacetamide (DMA), NP 40 (Nonidet P-40), and N-methyl-2-pyrrolidone (Pharmasolve),and amino acids.

27. The method of claim 21, wherein said surfactant is a nonionic surfactant.

28. The method of claim 27, wherein said nonionic surfactant is a polysorbate.

29. The method of claim 28, wherein said nonionic surfactant is polysorbate 20 or polysorbate 80.

30. The method of claim 21, wherein said stabilizer is selected from the group consisting of antioxidants and amino acids.

31. The method of claim 30, wherein said stabilizer is an antioxidant.

32. The method of claim 30, wherein said stabilizer is sodium metabisulfite.

33. The method of claim 21, wherein said method further comprises mixing a plurality of stabilizers.

34. The method of claim 33, wherein said stabilizers comprise L-arginine monohydrochloride, L-methionine, L-lysine monohydrochloride, and sodium metabisulfite.

35. The method of claim 21, wherein said bulking agent/tonicity adjuster is selected from the group consisting of mannitol, amino acids, lactose, potassium chloride, sodium chloride, maltose, sucrose, PEG's, trehalose, raffinose, dextrose, cyclodextrins, dextran, galacturonic acid, Ficoll, and polyvinylpyrrolidone (PVP).

36. The method of claim 35, wherein said bulking agent/tonicity adjuster is D-Mannitol.

37. The method of claim 21, wherein said chelator is pentetic acid (DTPA).

38. The method of claim 37, wherein said chelator is pentetic acid, said surfactant is polysorbate 20, said buffer is dibasic potassium phosphate, and said bulking agent/tonicity adjuster is D-mannitol.

39. The method of claim 21, wherein said formulation comprises about 0.0008 to 0.0012 mg/mL sincalide; about 20 to 50 mg/mL mannitol, about 2 to 7 mg/mL arginine; about 0.2 to 1 mg/mL methionine; about 2 to 30 mg/mL lysine; about 0.002 to 0.012 mg/mL sodium metabisulfite; about 0.000001 to 0.003 mg/mL polysorbate 20, about 0.1 to 3.0 mg/mL pentetic acid (DTPA); and about 1.1 to 1.8 mg/mL dibasic potassium phosphate.

40. A kit, comprising:

(i) a powder mixture comprising
  (a) sincalide,
  (b) at least one stabilizer,
  (c) a surfactant,
  (d) a chelator,
  (e) a bulking agent/tonicity adjuster, and
  (f) a buffer;

(ii) a container to hold said powder mixture; and

(iii) optionally, a physiologically acceptable fluid.

41. The kit of claim 40, wherein said buffer is selected from the group consisting of phosphoric acid, phosphate, citric acid, citrate, sulfosalicylate, acetic acid, acetate, methyl boronic acid, boronate, disodium succinate hexahydrate, one or more

amino acids, lactic acid, lactate, maleic acid, maleate, potassium chloride, benzoic acid, sodium benzoate, carbonic acid, carbonate, bicarbonate, boric acid, borate, sodium chloride, succinic acid, succinate, tartaric acid, tartrate, tris-(hydroxymethyl)aminomethane, and biological buffers.

42. The kit of claim 40, wherein said buffer is a phosphate buffer.

43. The kit of claim 40, wherein said buffer is dibasic potassium phosphate.

44. The kit of claim 40, wherein said surfactant is selected from the group consisting of anionic surfactants, pluronics, poloxamers, SDS, Triton-100, polysorbates, propylene glycol, PEG and similar compounds, Brij58 9poly(oxyethylene)20 cetyl ether, cremophor EL, cetyl trimethylammonium bromide (CTAB), dimethylacetamide (DMA), NP 40 (Nonidet P-40), and N-methyl-2-pyrrolidone (Pharmasolve),and amino acids.

45. The kit of claim 40, wherein said surfactant is a nonionic surfactant.

46. The kit of claim 45, wherein said nonionic surfactant is a polysorbate.

47. The kit of claim 46, wherein said nonionic surfactant is polysorbate 20 or polysorbate 80.

48. The kit of claim 40, wherein said stabilizer is selected from the group consisting of antioxidants and amino acids.

49. The kit of claim 48, wherein said stabilizer is an antioxidant.

50. The kit of claim 49, wherein said stabilizer is sodium metabisulfite.

51. The kit of claim 40, wherein said powder mixture comprises a plurality of stabilizers.

52. The kit of claim 51, wherein said stabilizers comprise L-arginine monohydrochloride, L-methionine, L-lysine monohydrochloride, and sodium metabisulfite.

53. The kit of claim 40, wherein said bulking agent/tonicity adjuster is selected from the group consisting of mannitol, amino acids, lactose, potassium chloride, sodium chloride, maltose, sucrose, PEG's, trehalose, raffinose, dextrose, cyclodextrins, dextran, galacturonic acid, Ficoll, and polyvinylpyrrolidone (PVP).

54. The kit of claim 40, wherein said chelator is pentetic acid (DTPA).

55. The kit of claim 40, wherein said container is a vial.

56. A method for treating or preventing a medical condition associated with total parenteral nutrition (TPN), said method comprising administering to a patient receiving TPN an effective amount of a sincalide formulation, said formulation comprising: (a) sincalide, (b) at least one stabilizer, (c) a surfactant, (d) a chelator, (e) a bulking agent/tonicity adjuster, and (f) a buffer.

57. The method of claim 56, wherein said medical condition is TPN-associated cholestatis.

58. The method of claim 56, wherein said formulation is administered by injection.

59. The method of claim 56, wherein said formulation has a pH from 6.0 to 8.0.

60. The method of claim 56, wherein said buffer is selected from the group consisting of phosphoric acid, phosphate, citric acid, citrate, sulfosalicylate, acetic acid, acetate, methyl boronic acid, boronate, disodium succinate hexahydrate, one or more amino acids, lactic acid, lactate, maleic acid, maleate, potassium chloride, benzoic acid, sodium benzoate, carbonic acid, carbonate, bicarbonate, boric acid, borate, sodium chloride, succinic acid, succinate, tartaric acid, tartrate, tris-(hydroxymethyl)aminomethane, and biological buffers.

61. The method of claim 56, wherein said buffer is a phosphate buffer.

62. The method of claim 61, wherein said buffer is dibasic potassium phosphate.

63. The method of claim 56, wherein said surfactant is selected from the group consisting of anionic surfactants, pluronics, poloxamers, SDS, Triton-100, polysorbates, propylene glycol, PEG and similar compounds, Brij58 9poly(oxyethylene)20 cetyl ether, cremophor EL, cetyl trimethylammonium bromide (CTAB), dimethylacetamide (DMA), NP 40 (Nonidet P-40), and N-methyl-2-pyrrolidone (Pharmasolve),and amino acids.

64. The method of claim 56, wherein said surfactant is a nonionic surfactant.

65. The method of claim 64, wherein said nonionic surfactant is a polysorbate.

66. The method of claim 65, wherein said nonionic surfactant is polysorbate 20 or polysorbate 80.

67. The method of claim 56, wherein said stabilizer is selected from the group consisting of antioxidants and amino acids.

68. The method of claim 67, wherein said stabilizer is an antioxidant.

69. The method of claim 68, wherein said stabilizer is sodium metabisulfite.

70. The method of claim 56, wherein said method comprises mixing a plurality of stabilizers.

71. The method of claim 69, wherein said stabilizers comprise L-arginine monohydrochloride, L-methionine, L-lysine monohydrochloride, and sodium metabisulfite.

72. The method of claim 56, wherein said bulking agent/tonicity adjuster is selected from the group consisting of mannitol, amino acids, lactose, potassium chloride, sodium chloride, maltose, sucrose, PEG's, trehalose, raffinose, dextrose, cyclodextrins, dextran, galacturonic acid, Ficoll, and polyvinylpyrrolidone (PVP).

73. The method of claim 72, wherein said bulking agent/tonicity adjuster is D-mannitol.

74. The method of claim 56 wherein said chelator is pentetic acid (DTPA).

75. The method of claim 74 wherein said chelator is pentetic acid, said surfactant is polysorbate 20, said buffer is dibasic potassium phosphate, and said bulking agent/tonicity adjuster is D-mannitol.

76. The method of claim 56, wherein said formulation comprises about 0.0008 to 0.0012 mg/mL sincalide; about 20 to 50 mg/mL D-mannitol, about 2 to 7 mg/mL L-arginine; about 0.2 to 1 mg/mL L-methionine; about 2 to 30 mg/mL L-lysine; about 0.002 to 0.012 mg/mL sodium metabisulfite; about 0.000001 to 0.003 mg/mL polysorbate 20, about 0.1 to 3 mg/mL pentetic acid (DTPA); and about 1.1 to 1.8 mg/mL dibasic potassium phosphate.

77. A method for imaging the hepatobiliary system of a subject, said method comprising:

(a) administering a hepatobiliary imaging agent to said subject;

(b) before or after step (a), administering to a subject a sincalide formulation comprising: (i) sincalide, (ii) at least one stabilizer, (iii) a surfactant, (iv) a chelator, (v) a bulking agent/tonicity adjuster, and (vi) a buffer; and

(c) detecting said imaging agent in said subject with a detection device.

78. The method of claim 77, wherein said sincalide formulation is administered by injection.

79. The method of claim 77, wherein said sincalide formulation is administered to said subject before administration of said hepatobiliary imaging agent.

80. The method of claim 77, wherein said sincalide formulation is administered to said subject after administration of said hepatobiliary imagining agent.

81. The method of claim 77, wherein said hepatobiliary imaging agent is a ^{99m}Tc-IDA (Iminodiacetic acid) analog.

82. The method of claim 77, wherein said hepatobiliary imaging agent is ^{99m}Tc-mebrofenin.

83. The method of claim 77, wherein said method further comprises, after administration of said sincalide formulation, measuring said the gallbladder ejection fraction (GBEF) of said subject.

84. The method of claim 77, wherein said detection device scans the body of said subject for radioactivity.

85. The method of claim 84, wherein said detection device is a gamma camera.

86. The method of claim 77, wherein said formulation has a pH from 6.0 to 8.0.

87. The method of claim 77, wherein said buffer is selected from the group consisting of phosphoric acid, phosphate, citric acid, citrate, sulfosalicylate, acetic acid, acetate, methyl boronic acid, boronate, disodium succinate hexahydrate, one or more amino acids, lactic acid, lactate, maleic acid, maleate, potassium chloride, benzoic acid, sodium benzoate, carbonic acid, carbonate, bicarbonate, boric acid, borate, sodium chloride, succinic acid, succinate, tartaric acid, tartrate, tris-(hydroxymethyl)aminomethane, and biological buffers.

88. The method of claim 77, wherein said buffer is a phosphate buffer.

89. The method of claim ~~88,~~ wherein said buffer is dibasic potassium phosphate.

90. The method of claim 77, wherein said surfactant is selected from the group consisting of anionic surfactants, pluronics, poloxamers, SDS, Triton-100, polysorbates, propylene glycol, PEG and similar compounds, Brij58 9poly(oxyethylene)20 cetyl ether, cremophor EL, cetyl trimethylammonium bromide (CTAB), dimethylacetamide (DMA), NP 40 (Nonidet P-40), and N-methyl-2-pyrrolidone (Pharmasolve),and amino acids.

91. The method of claim 77, wherein said surfactant is a nonionic surfactant.

92. The method of claim 91, wherein said nonionic surfactant is a polysorbate.

93. The method of claim 92, wherein said nonionic surfactant is polysorbate 20 or polysorbate 80.

94. The method of claim ~~77~~, wherein said stabilizer is selected from the group consisting of antioxidants and amino acids.

95. The method of claim 94, wherein said stabilizer is an antioxidant.

96. The method of claim 95, wherein said stabilizer is sodium metabisulfite.

97. The method of claim 77, wherein said method comprises mixing a plurality of stabilizers.

98. The method of claim 97, wherein said stabilizers comprise L-arginine monohydrochloride, L-methionine, L-lysine monohydrochloride, and sodium metabisulfite.

99. The method of claim 77, wherein said bulking agent/tonicity adjuster is selected from the group consisting of mannitol, amino acids, lactose, potassium chloride, sodium chloride, maltose, sucrose, PEG's, trehalose, raffinose, dextrose, cyclodextrins, dextran, galacturonic acid, Ficoll, and polyvinylpyrrolidone (PVP).

100. The method of claim 77, wherein said bulking agent/tonicity adjuster is D-mannitol.

101. The method of claim 77, wherein said chelator is pentetic acid (DTPA).

102. The method of claim 101, wherein said chelator is pentetic acid, said surfactant is polysorbate 20, said buffer is dibasic potassium phosphate, and said bulking agent/tonicity adjuster is D-mannitol.

103. The method of claim 77, wherein said formulation comprises about 0.0008 to 0.0012 mg/mL sincalide; about 20 to 50 mg/mL D-mannitol, about 2 to 7 mg/mL L-arginine; about 0.2 to 1 mg/mL L-methionine; about 2 to 30 mg/mL L-

lysine; about 0.002 to 0.012 mg/mL Sodium metabisulfite; about 0.000001 to 0.003 mg/mL polysorbate 20; about 0.1 to 3 mg/mL pentetic acid (DTPA); and about 1.1 to 1.8 mg/mL dibasic potassium phosphate.

104. A method for imaging the hepatobiliary system of a subject, said method comprising:

a) administering to a subject a sincalide formulation comprising: (i) sincalide, (ii) at least one stabilizer, (iii) a surfactant, (iv) a chelator, (v) a bulking agent/tonicity adjuster, and (vi) a buffer; and

b) scanning the subject using a diagnostic imaging modality.

105. The method of claim 104 wherein said imaging modality is selected from the group consisting of magnetic resonance imaging, scintigraphic imaging and ultrasound imaging.

106. A stabilized, physiologically acceptable formulation of sincalide comprising:

(a) about 0.001 mg/mL sincalide;
(b) about 34 mg/mL D-mannitol;
(c) about 6 mg/mL L-arginine;
(d) about 0.8 mg/mL L-methionine;
(e) about 3 mg/mL L-lysine;
(f) about 0.008 mg/mL sodium metabisulfite;
(g) less than about 0.01 mg/mL polysorbate 20;
(h) about 0.4 mg/mL pentetic acid; and
(i) about 1.8 mg/mL dibasic potassium phosphate.

107. The kit of claim 40 comprising:

a) about 0.005 mg sincalide;
b) about 100 to 250 mg mannitol;
c) about 0.000005 to 0.015 mg polysorbate 20;

d) about 2 mg pentetic acid (DTPA);
e) about 0.01 to 0.06 mg sodium metabisulfite;
f) about 5.4 to 12 mg potassium phosphate (dibasic);
g) about 1 to 5 mg methionine;
h) about 10 to 60 mg lysine; and
i) about 10 to 35 mg arginine.

108. A kit comprising:

a) about 0.005 mg sincalide;
b) about 170 mg D-mannitol;
c) less than about 0.01 mg polysorbate 20;
d) about 2 mg DTPA;
e) about 0.04 mg sodium metabisulfite;
f) about 9 mg potassium phosphate (dibasic);
g) about 4 mg L-methionine;
h) about 15 mg L-lysine monohydrochloride; and
i) about 30 mg L-arginine monohydrochloride.

SINCALIDE FORMULATIONS

ABSTRACT

The invention features sincalide formulations that include an effective amount of sincalide, a bulking agent/tonicity adjuster, a stabilizer, a surfactant, a chelator, and a buffer. The invention also features kits and methods for preparing improved sincalide formulations, as well as methods for treating, preventing, and diagnosing gall bladder-related disorders using sincalide formulations.

PATENT
ATTORNEY DOCKET NO: 50203/017001

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled SINCALIDE FORMULATIONS, the specification of which

- ☒ is attached hereto.
- ☐ was filed on _____ as Application Serial No. _____ and was amended on _____.
- ☐ was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

FOREIGN PRIORITY RIGHTS: I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
			Yes/No

PROVISIONAL PRIORITY RIGHTS: I hereby claim priority benefits under Title 35, United States Code, § 119(e) and § 120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status

NON-PROVISIONAL PRIORITY RIGHTS: I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all

COMBINED DECLARATION AND POWER OF ATTORNEY

information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bieker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Vicki Healy, Reg. No. 48,343.

Address all telephone calls to: Karen L. Elbing, Ph.D. at 617/428-0200.

Address all correspondence to: Karen L. Elbing, Ph.D., at Clark & Elbing LLP, 101 Federal Street, Boston, MA 02110. **Customer No: 21559**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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Signature:			Date:

COMBINED DECLARATION AND POWER OF ATTORNEY

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Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Ernst Schramm	North Brunswick, NJ	815 Prospect Avenue North Brunswick, NJ	GERMAN
Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Gregory W. White	Monmouth Junction, NJ	23 Oaktree Road Monmouth Junction, NJ	US
Signature:			Date:

COMBINED DECLARATION AND POWER OF ATTORNEY

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Julius P. Zodda	Mercerville, NJ	3 Tigers Court Mercerville, NJ	US
Signature:			Date:

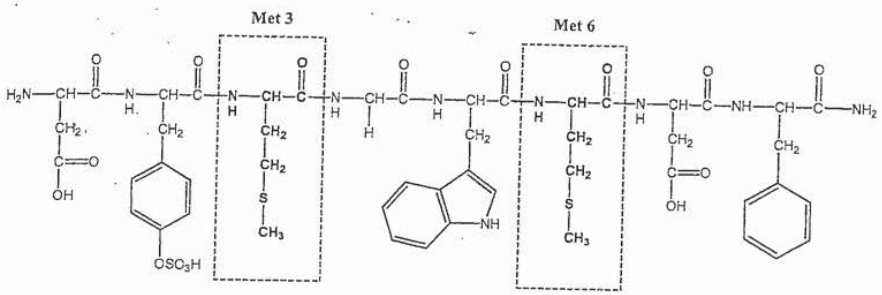


FIG. 1

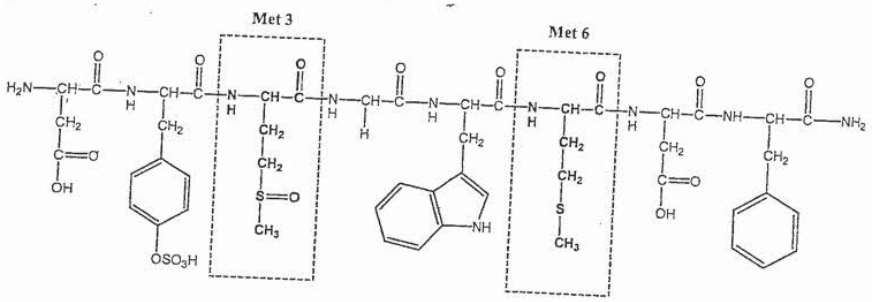


FIG. 2

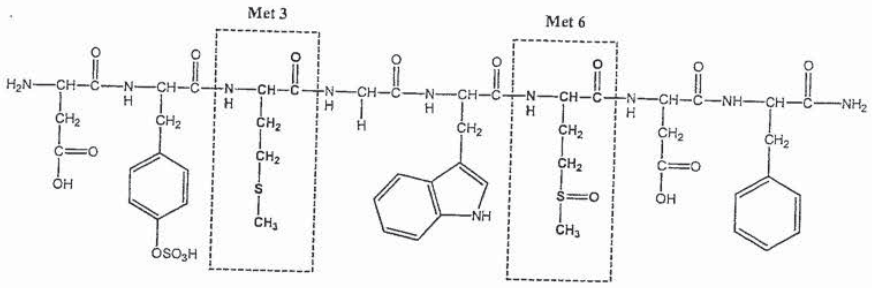


FIG. 3

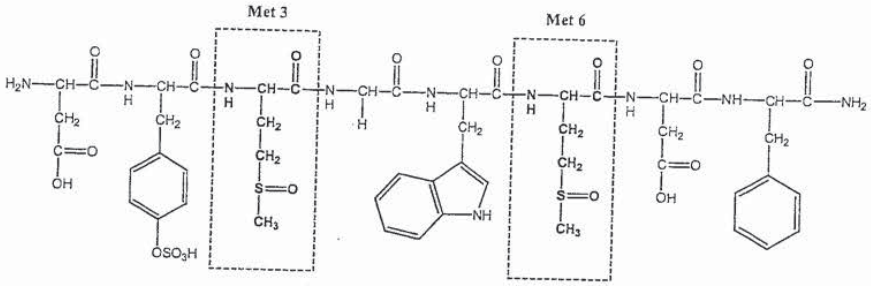


FIG. 4

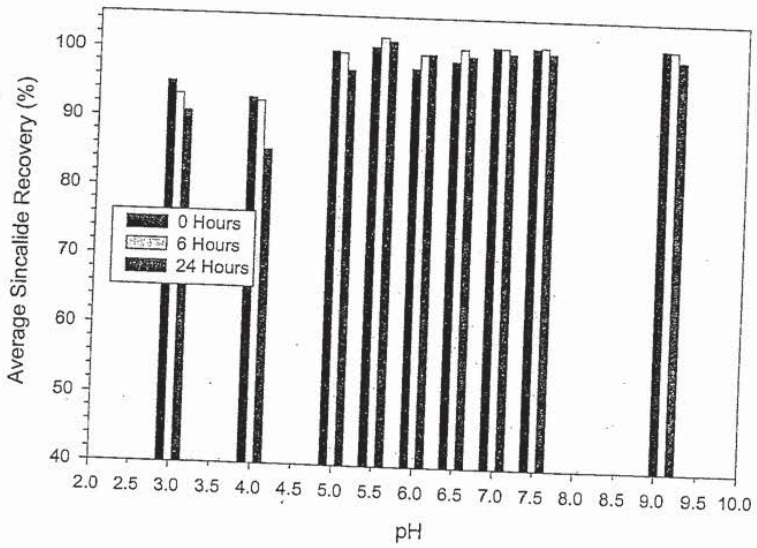


FIG. 5

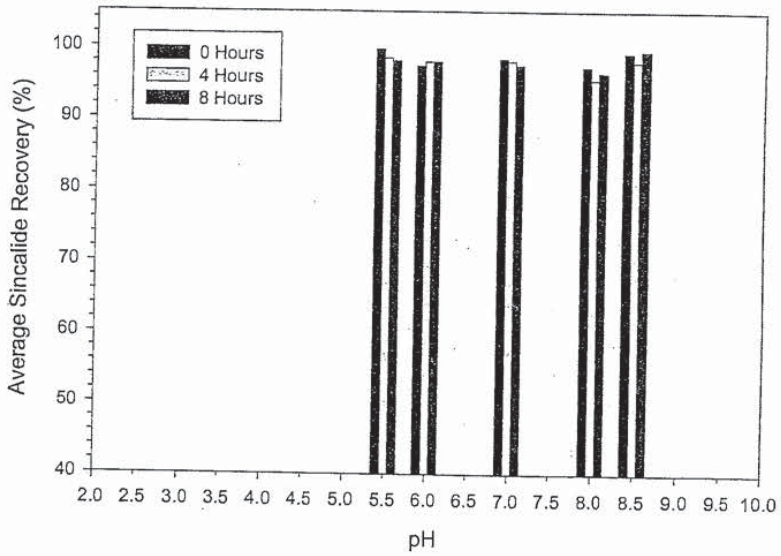


FIG. 6

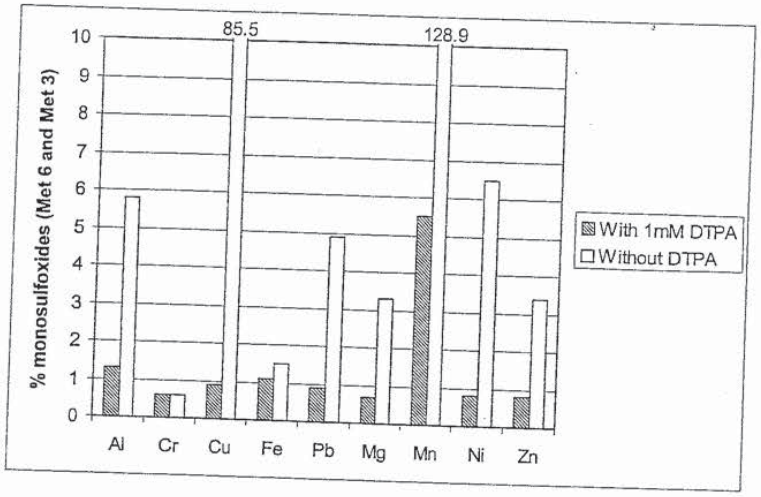
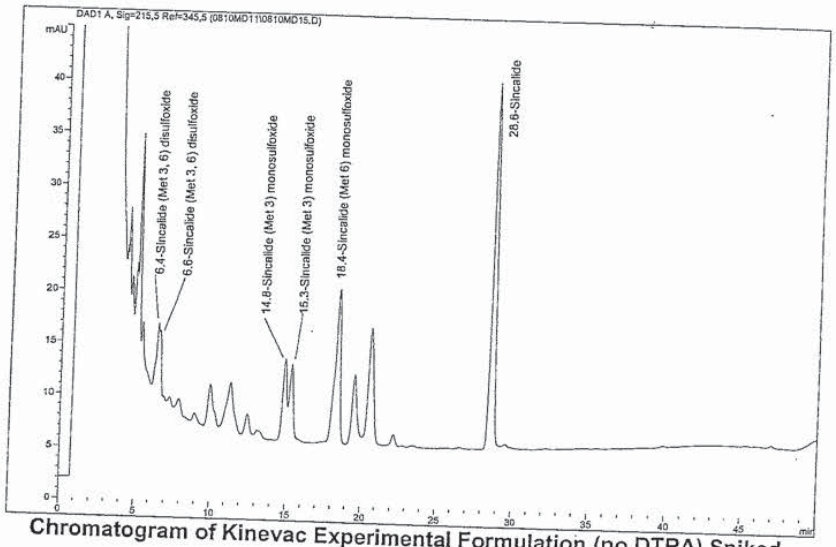


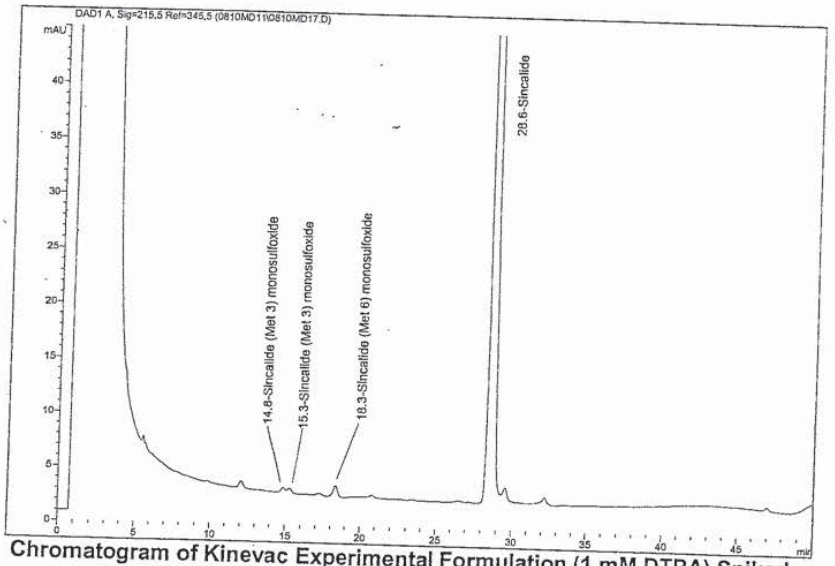
FIG. 7



Chromatogram of Kinevac Experimental Formulation (no DTPA) Spiked with 0.63 mM Cu^{2+}

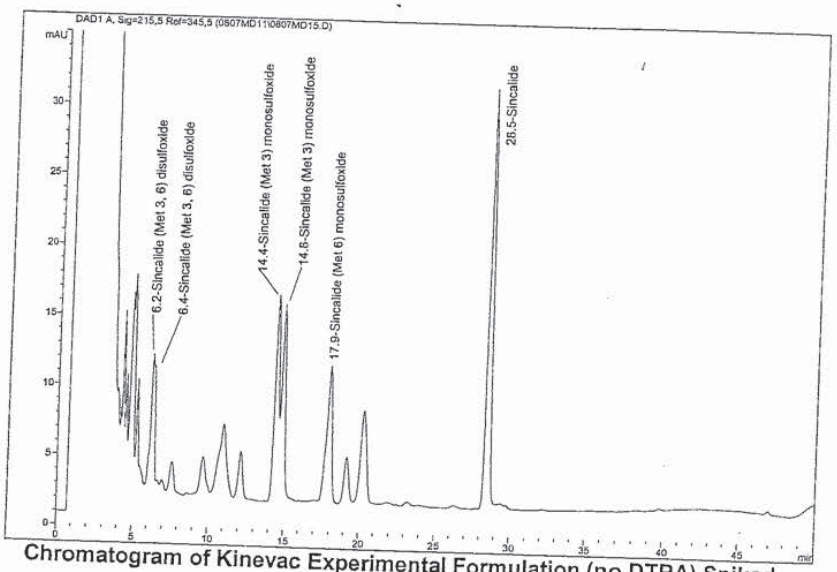
FIG. 8

PRINT OF DRAWINGS
AS ORIGINALLY FY



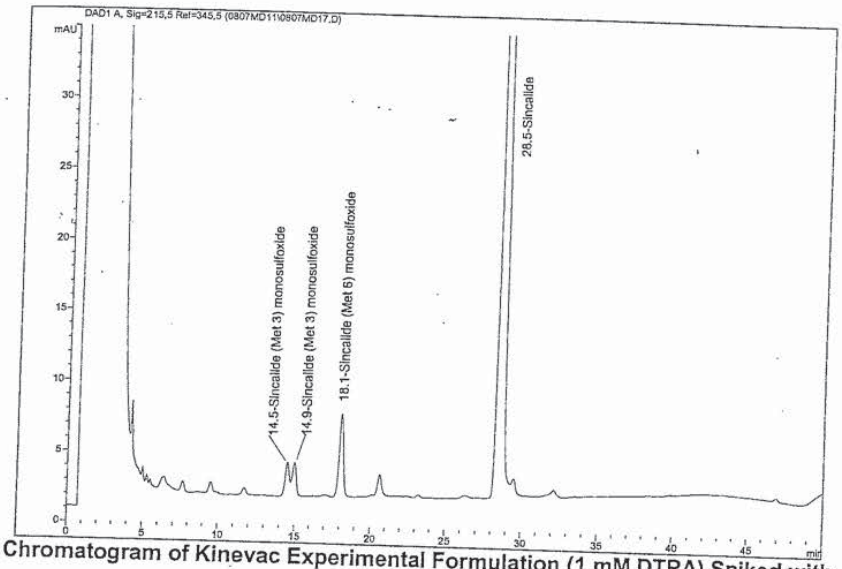
Chromatogram of Kinevac Experimental Formulation (1 mM DTPA) Spiked with 0.63 mM Cu^{2+}

FIG. 9



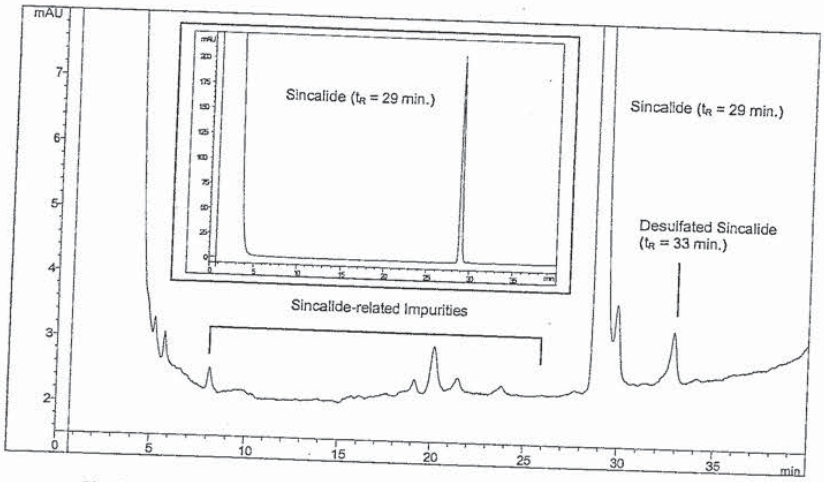
Chromatogram of Kinevac Experimental Formulation (no DTPA) Spiked with 0.18 mM Mn^{2+}

FIG. 10



Chromatogram of Kinevac Experimental Formulation (1 mM DTPA) Spiked with 0.18 mM Mn^{2+}

FIG. 11



Typical Full-Scale and Expanded-Scale Chromatograms of Reconstituted Kinevac

FIG. 12

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

35743 7590 08/01/2003

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
GEORGE, KONATA M	

ART UNIT	PAPER NUMBER
1616	

DATE MAILED: 08/01/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 07-01)

DETAILED ACTION

Claims 1-108 are pending in this application.

Restriction Requirement

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-39, 56-76 and 106, drawn to a drug composition and method of use, classified in class 424, subclass 400.

II. Claims 40-55, 107-108, drawn to a composition comprising a kit, classified in class 428, subclass 34.1.

III. Claims 77-105, drawn to method for imaging the hepatobiliary system, classified in class 424, subclass 1.65.

The inventions are distinct, each from the other because:

Invention III is unrelated to inventions II and I. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case the different inventions group III is unrelated to groups I and II as group III is directed towards a method for imaging the hepatobiliary system by administering a hepatobiliary imaging agent to a subject and groups I and II are directed to a stabilized, physiologically acceptable formulations of sincalide.

Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different

product or (2) the product as claimed can be used in a materially different process of using that product (MPEP § 806.05(h)). In the instant case the product as claimed can be used in a materially different process such as formulated into a tablet, capsule, etc.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art because of their recognized divergent subject matter, restriction for examination purposes as indicated is proper.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Telephone Inquiries

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Konata M. George, whose telephone number is (703) 308-4646. The examiner can normally be reached from 8AM to 5:30PM Monday to Thursday, and on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Thurman Page, can be reached at (703) 308-2927. The fax phone numbers

for the organization where this application or proceeding is assigned are (703) 308-4556 for regular communications and for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-1235.

Konata M. George
Patent Examiner
Art Unit 1616

Office Action Summary	Application No.	Applicant(s)	
	10/222,540	METCALFE ET AL.	
	Examiner	Art Unit	
	Konata M. George	1616	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-108 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) 1-108 are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

Customer No. 35743

Attorney Docket No.: 057637/01160

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	Metcalfe, Edmund et al.	Confirmation No. 6555
Serial No.	10/222,540	
Filed	August 16, 2002	
For	**SINCALIDE FORMULATIONS**	
Group Art Unit	1619	
Examiner	George, Konata M.	

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited on August 27, 2003, with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Arlington, VA 22313-1450.

Signature: C. Caggiano

Carrie Caggiano
Kramer Levin Naftalis & Frankel LLP

Commissioner for Patents
P.O. Box 1450
Arlington, VA 22313-1450

August 27, 2003

RESPONSE TO RESTRICTION REQUIREMENT

This is being filed in response to the Office Action mailed August 1, 2003 ("Office Action"). Since a response is due September 2, 2003, this Response is timely filed.

Remarks begin on page 2 of this paper.

KL3 2286529.1

REMARKS

In response to the Office Action of August 1, 2003, restriction to one of the following inventions is required under 35 U.S.C. § 121:

I. Claims 1-39, 56-76 and 106, drawn to a drug composition and method of use, classified in class 424, subclass 400.

II. Claims 40-55, 107-108, drawn to a composition comprising a kit, classified in class 428, subclass 34.1.

III. Claims 77-105, drawn to method for imaging the hepatobiliary system, classified in class 424, subclass 1.65.

Applicants wish to thank the Examiner for the courtesies extended during a telephone interview on August 25, 2003 in which the Examiner agreed to withdraw the restriction requirement as between Group I and Group II. As such, all of the claims of Group I and II are now combined.

Therefore, Applicants elect the combined Group I and Group II claims, namely claims 1-39-76 and 106-108, drawn to a drug composition, method of use, and composition comprising a kit, for prosecution on the merits.

Favorable consideration of the application and prompt issuance of a Notice of Allowance are earnestly solicited.

No additional fee is required. If there any such fees, please charge them to our firm Deposit Account No. 50-0540.

Respectfully submitted,

August 27, 2003

By: _____

Donald Rhoads, Reg. No. 34,705
Albert B. Chen, Reg. No. 41,667
Attorney for Applicants
KRAMER LEVIN NAFTALIS & FRANKEL LLP
919 Third Avenue
New York, New York 10022
(212) 715-9100 (phone)
(212) 715-8000 (fax)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

35743 7590 10/03/2003

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
GEORGE, KONATA M	
ART UNIT	PAPER NUMBER
1616	

DATE MAILED: 10/03/2003

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

TITLE OF INVENTION: SINCALIDE FORMULATIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	01/05/2004

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE REFLECTS A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE APPLIED IN THIS APPLICATION. THE PTOL-85B (OR AN EQUIVALENT) MUST BE RETURNED WITHIN THIS PERIOD EVEN IF NO FEE IS DUE OR THE APPLICATION WILL BE REGARDED AS ABANDONED.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:
A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status is changed, pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above and notify the United States Patent and Trademark Office of the change in status, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check the box below and enclose the PUBLICATION FEE and 1/2 the ISSUE FEE shown above.

☐ Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 10/03) Approved for use through 04/30/2004.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

or Fax **(703) 746-4000**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

35743 7590 10/03/2003

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

TITLE OF INVENTION: SINCALIDE FORMULATIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	01/05/2004

EXAMINER	ART UNIT	CLASS-SUBCLASS
GEORGE, KONATA M	1616	424-489000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the USPTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent); ❑ individual ❑ corporation or other private group entity ❑ government

4a. The following fee(s) are enclosed:

❑ Issue Fee

❑ Publication Fee

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s):

❑ A check in the amount of the fee(s) is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

Director for Patents is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

(Authorized Signature) (Date)

NOTE; The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE(S)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

35743 7590 10/03/2003

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
GEORGE, KONATA M	

ART UNIT	PAPER NUMBER
1616	

DATE MAILED: 10/03/2003

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) system (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (703) 305-1383. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 10/03) Approved for use through 04/30/2004.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

35743 7590 10/03/2003

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
GEORGE, KONATA M	

ART UNIT	PAPER NUMBER
1616	

DATE MAILED: 10/03/2003

Notice of Fee Increase on October 1, 2003

If a reply to a "Notice of Allowance and Fee(s) Due" is filed in the Office on or after October 1, 2003, then the amount due will be higher than that set forth in the "Notice of Allowance and Fee(s) Due" since there will be an increase in fees effective on October 1, 2003. See Revision of Patent Fees for Fiscal Year 2004; Final Rule, 68 Fed. Reg. 41532, 41533, 41534 (July 14, 2003).

The current fee schedule is accessible from (http://www.uspto.gov/main/howtofees.htm).

If the fee paid is the amount shown on the "Notice of Allowance and Fee(s) Due" but not the correct amount in view of the fee increase, a "Notice of Pay Balance of Issue Fee" will be mailed to applicant. In order to avoid processing delays associated with mailing of a "Notice of Pay Balance of Issue Fee," if the response to the Notice of Allowance is to be filed on or after October 1, 2003 (or mailed with a certificate of mailing on or after October 1, 2003), the issue fee paid should be the fee that is required at the time the fee is paid. If the issue fee was previously paid, and the response to the "Notice of Allowance and Fee(s) Due" includes a request to apply a previously-paid issue fee to the issue fee now due, then the difference between the issue fee amount at the time the response is filed and the previously-paid issue fee should be paid. See Manual of Patent Examining Procedure, Section 1308.01 (Eighth Edition, August 2001).

Effective October 1, 2003, 37 CFR 1.18 is amended by revising paragraphs (a) through (c) to read as set forth below.

Section 1.18 Patent post allowance (including issue) fees.

(a) Issue fee for issuing each original or reissue patent, except a design or plant patent:
By a small entity (Sec. 1.27(a)) $665.00
By other than a small entity $1,330.00

(b) Issue fee for issuing a design patent:
By a small entity (Sec. 1.27(a)) $240.00
By other than a small entity $480.00

(c) Issue fee for issuing a plant patent:
By a small entity (Sec. 1.27(a)) $320.00
By other than a small entity $640.00

Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 10/03) Approved for use through 04/30/2004.

Notice of Allowability	Application No.	Applicant(s)	
	10/222,540	METCALFE ET AL.	
	Examiner	Art Unit	
	Konata M. George	1616	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *Election of Spec. September 2, 2003*.
2. ☒ The allowed claim(s) is/are *1-108*.
3. ☒ The drawings filed on *16 August 2002* are accepted by the Examiner.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.
5. ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
   (a) ☐ The translation of the foreign language provisional application has been received.
6. ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application. **THIS THREE-MONTH PERIOD IS NOT EXTENDABLE**

7. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

8. ☐ CORRECTED DRAWINGS must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No. ____.
   (b) ☐ including changes required by the proposed drawing correction filed _____, which has been approved by the Examiner.
   (c) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No. _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet.

9. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1☒ Notice of References Cited (PTO-892)
2☐ Notice of Informal Patent Application (PTO-152)
3☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
4☐ Interview Summary (PTO-413), Paper No.____ .
5☐ Information Disclosure Statements (PTO-1449), Paper No. ____.
6☒ Examiner's Amendment/Comment
7☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
8☒ Examiner's Statement of Reasons for Allowance
9☐ Other .

THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

DETAILED ACTION

Claims 1-108 are pending in this application.

Drawings

1. The examiner under 37 CFR 1.184 or 1.152 accepts the drawing(s) filed August 16, 2002.

Restriction Requirement

2. Claims 1-55 and 106-108 are directed to an allowable product. Pursuant to the procedures set forth in the Official Gazette notice dated March 26, 1996 (1184 O.G. 86), claims 77-105, directed to the process of making or using the patentable product, previously withdrawn from consideration as a result of a restriction requirement, are now subject to being rejoined. Claims 77-105 are hereby rejoined and fully examined for patentability under 37 CFR 1.104.

Since all claims previously withdrawn from consideration under 37 CFR 1.142 have been rejoined, the restriction requirement made in Paper No. 4 is hereby withdrawn.

Examiner's Amendment

3. An examiner's amendment to the record appears below. Should that changes and/or additions be unacceptable to applicant, an amendment may be filed as provided

by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Mr. Albert Chen on September 17, 2003.

Amend the claims as follows:

IN THE CLAIMS:

In claim 56, line 1 delete "or preventing".

Statement of Reasons for Allowance

4. The following is an examiner's statement of reasons for allowance:

The claims are allowable over the prior art because the prior art does not teach, disclose nor make obvious the invention as claimed. The closet prior is that of Wang et al. (US 5,011,678) disclosed within. It is taught in Wang a composition comprising pharmaceutically active agents such as sincalide (col. 7, line 20) a biocompatible amphiphilac steroid and a biocompatible (hydro/fluoro) carbon propellant. The composition does not contain additional ingredients as claimed by applicant such as a stabilizer, surfactant/solubilizer or chelator.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

5. Claims 1-108 are allowed.

Telephone Inquiries

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Konata M. George, whose telephone number is (703) 308-4646. The examiner can normally be reached from 8AM to 5:30PM Monday to Thursday, and on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Thurman Page, can be reached at (703) 308-2927. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4556 for regular communications and for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-1235.

Konata M. George
kmg

THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Notice of References Cited

Application/Control No.	Applicant(s)/Patent Under Reexamination	
10/222,540	METCALFE ET AL.	
Examiner	Art Unit	Page 1 of 1
Konata M. George	1616	

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,011,678	04-1991	Wang et al.	424/45
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

KCG

PTO/SB/30 (10-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION (RCE) TRANSMITTAL

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	10/222,540
Filing Date	August 16, 2002
First Named Inventor	Metcalfe, Edmund
Art Unit	1616
Examiner Name	Konata, G.
Attorney Docket Number	57637/1160

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114
   - a. ☐ Previously submitted
     - i. ☐ Consider the amendment(s)/reply under 37 CFR 1.116 previously filed on _____ (Any unentered amendment(s) referred to above will be entered).
     - ii. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
     - iii. ☐ Other _____
   - b. ☒ Enclosed
     - i. ☐ Amendment/Reply
     - ii. ☐ Affidavit(s)/Declaration(s)
     - iii. ☒ Information Disclosure Statement (IDS)
     - iv. ☒ Other PTO Form-1449

2. Miscellaneous
   - a. ☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)
   - b. ☐ Other _____

3. Fees — The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   - a. ☒ A check in the amount of $770.00 is enclosed
   - b. ☒ The Director is hereby authorized to charge any fee deficiency, or credit any overpayments, to Deposit Account No. 50-0540
   - c. ☐ Payment by credit card (Form PTO-2038 enclosed)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Name (Print/Type)	Albert B. Chen	Registration No. (Attorney/Agent)	41,667
Signature		Date	December 19, 2003

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.

Name (Print/Type)	Carrie L. Caggiano		
Signature		Date	December 19, 2003

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND Fees and Completed Forms to the following address: Assistant Commissioner for Patents, Washington, DC 20231.

12/24/2003 EFLORES 00000070 10222540
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KL3:2310318.1

Match and Return

Customer No. 35743 Attorney Docket No. 57637/1160

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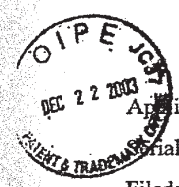
7/20/0



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Metcalfe et al.
Serial No.:	10/222,540
Filed:	August 16, 2002
Group Art Unit:	1616
Examiner:	Konata M. George
For:	**SINACALIDE FORMULATIONS**

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to, Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on:

Date: 12-19-03

Signature: C. Caggiano
Carrie L. Caggiano

INFORMATION DISCLOSURE STATEMENT

Sir:

Applicants respectfully submit this Information Disclosure Statement pursuant to 37 C.F.R. §§ 1.97 and 1.98 in order to comply with the duty of disclosure under 37 C.F.R. § 1.56. These references are listed on the attached modified PTO Form No. 1449.

A copy of the listed references is enclosed for the convenience of the Examiner.

This Information Disclosure Statement is being filed concurrently with a Request For Continued Examination and before the mailing of a first Office Action on the merits after the filing of an RCE, and thus, pursuant to 37 C.F.R. § 1.97(b)(4), Applicants respectfully request that the information be expressly considered during the prosecution of this application and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom. Applicants further request that a copy of the PTO Form No. 1449, appropriately initialed by the Examiner, be returned to Applicants' attorney.

KLJ:2310285.1

It is believed that no fees are due in connection with this Information Disclosure Statement. However, should any fees be due, the commissioner is authorized to charge Deposit Account No. 50-0540 for such fees. Early and favorable action is earnestly solicited.

Dated: December 19, 2003

Respectfully submitted,

Donald Rhoads, Reg. No. 34,705
Albert B. Chen, Reg. No. 41,667
Attorneys for Applicant
KRAMER LEVIN NAFTALIS & FRANKEL LLP
919 Third Avenue
New York, New York 10022
(212) 715-9100

KL3:2310285.1

Based on Form PTO-1449 (3/90)	ATTY. DOCKET NO. 57637/1160	SERIAL NO. 10/222,540
LIST OF REFERENCES CITED BY APPLICANT (Use several sheets if necessary)	**APPLICANTS** Metcalfe et al.	
	FILING DATE August 16, 2002	**GROUP ART UNIT** 1616

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
KG	AA	5,011,678	4/30/91	Wang et al.	424	65	
	AB	6,306,905B1	10/23/01	Kurz et al.	514	551	
	AC	3,723,406	3/27/73	Ondetti et al.	260	112.5	
	AD	6,326,406B1	12/4/01	De Tommaso	514	731	
	AE	6,358,528B1	3/19/02	Grimmett et al.	424	474	
	AF	5,833,948	11/10/98	Tournier et al.	424	9.321	
	AG	5,567,414	10/22/96	Schneider et al.	424	9.52	
	AH	6,110,443	8/29/00	Schneider et al.	424	9.51	
KG	AI	5,556,610	9/17/96	Yan et al.	424	9.52	
	AJ						
	AK						
	AL						
	AM						
	AN						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	TRANSLATION	
	BA							
	BB							
	BC							
	BD							
	BE							

OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)

KG	CA	Sitzmann, et al., "Cholecystokinin Prevents Parenteral Nutrition Induced Biliary Sludge in Humans," Surgery, Gynecology & Obstetrics, Vol. 170, January 1990, pp. 25-31
	CB	Teitelbaum et al., "Treatment of Parenteral Nutrition-Associated Cholestasis with Cholecystokinin-Octapeptide," Journal of Pediatric Surgery, Vol. 30, No. 7, July 1995, pp. 1082-1085
	CC	Moss and Amii, "New Approaches to Understanding the Etiology and Treatment of Total Parenteral Nutrition-Associated Cholestasis," Seminars in Pediatric Surgery, Vol. 8, No. 3, August 1999, pp. 140-147
	CD	Teitelbaum, "Parenteral Nutrition-Associated Cholestasis," Current Opinion in Pediatrics, Vol. 9, 1997, pp. 270-275
	CE	Teitelbaum and Tracy, "Parenteral Nutrition-Associated Cholestasis," Seminars in Pediatric Surgery, Vol. 10, No. 2, May 2001, pp. 72-80
	CF	Strickley, "Parenteral Formulations of Small Molecules Therapeutics Marketed in the United States (1999) – Part 1," PDA Journal of Pharmaceutical Science & Technology, Vol. 53, No. 6, November-December 1999, pp. 324-349
	CG	Strickley, "Parenteral Formulations of Small Molecules Therapeutics Marketed in the United States (1999) – Part II," PDA Journal of Pharmaceutical Science & Technology, Vol. 54, No. 1, January-February 2000, pp. 69-96
	CH	Strickley, "Parenteral Formulations of Small Molecules Therapeutics Marketed in the United States (1999) – Part III," PDA Journal of Pharmaceutical Science & Technology, Vol. 54, No. 2, March-April 2000, pp. 152-169
KG	CI	Nema et al., "Excipients and Their Use in Injectable Products," PDA Journal of Pharmaceutical Science & Technology, Vol. 51, No. 4, July-August 1997, pp. 166-171

BRACCO DIAGNOSTICS™

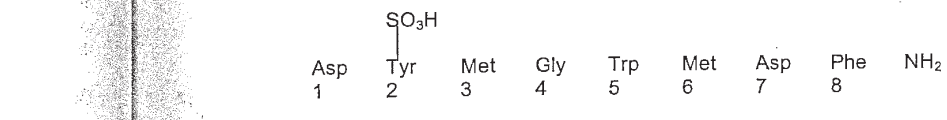
J3-398J

CAUTION: Federal law prohibits dispensing without prescription.

KINEVAC®
Sincalide for Injection

DESCRIPTION

Kinevac (Sincalide for Injection) is a cholecystopancreatic-gastrointestinal hormone peptide for parenteral administration. The agent is a synthetically-prepared C-terminal octapeptide of cholecystokinin. Each vial of sincalide provides a sterile nonpyrogenic lyophilized white powder consisting of 5 mcg sincalide with 45 mg sodium chloride to provide tonicity; sodium hydroxide or hydrochloric acid may have been added prior to lyophilization for pH adjustment (5.5 to 6.5). At the time of manufacture, the air in the vial is replaced with nitrogen. Sincalide is designated chemically as L-aspartyl-L-tyrosyl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartylphenyK-alaninamide hydrogen sulfate (ester). Graphic formula:



$C_{49}H_{62}N_{10}O_{16}S_3$ MW 1143.27 CAS-25126-32-3

CLINICAL PHARMACOLOGY

When injected intravenously, sincalide produces a substantial reduction in gallbladder size by causing this organ to contract. The evacuation of bile that results is similar to that which occurs physiologically in response to endogenous cholecystokinin. The intravenous (bolus) administration of sincalide causes a prompt contraction of the gallbladder that becomes maximal in 5 to 15 minutes, as compared with the stimulus of a fatty meal which causes a progressive contraction that becomes maximal after approximately 40 minutes. Generally, a: 40 percent reduction in radiographic area of the gallbladder is considered satisfactory contraction, although some patients will show area reduction of 60 to 70 percent.

Like cholecystokinin, sincalide stimulates pancreatic secretion; concurrent administration with secretin increases both the volume of pancreatic secretion and the output of bicarbonate and protein (enzymes) by the gland. This combined effect of secretin and sincalide permits the assessment of specific pancreatic function through measurement and analysis of the duodenal aspirate. The parameters usually determined are: volume of the secretion; bicarbonate concentration; and amylase content (which parallels the content of trypsin and total protein).

Both cholecystokinin and sincalide stimulate intestinal motility, and may cause pyloric contraction which retards gastric emptying.

INDICATIONS AND USAGE

Kinevac (Sincalide for Injection) may be used: (1) to stimulate gallbladder contraction, as may be assessed by contrast agent cholecystography or ultrasonography, or to obtain by duodenal aspiration a sample of concentrated bile for analysis of cholesterol, bile salts, phospholipids, and crystals; (2) to stimulate pancreatic secretion (especially in conjunction with secretin) prior to obtaining a duodenal aspirate for analysis of enzyme activity, composition, and cytology; (3) to accelerate the transit of a barium meal through the small bowel, thereby decreasing the time and-extent of radiation associated with fluoroscopy and x-ray examination of the intestinal tract.

CONTRAINDICATIONS

The preparation is contraindicated in patients hypersensitive to sincalide and in patients with intestinal obstruction.

WARNINGS

Because of Kinevac's effect on smooth muscle, pregnant patients should be advised that spontaneous abortion or premature induction of labor may occur (see Pregnancy Category B).

PRECAUTIONS

General

The possibility exists that stimulation of gallbladder contraction in patients with small gallbladder stones could lead to the evacuation of the stones from the gallbladder, resulting in their lodging in the cystic duct or in the common bile duct. The risk of such an event is considered to be minimal because sincalide, when given as directed, does not ordinarily cause complete contraction of the gallbladder.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential, or possible impairment of fertility in males or females.

Teratogenic Effects

Pregnancy Category B

Reproduction studies in rats in which sincalide was administered subcutaneously at doses up to 12.5 times the maximum recommended human dose revealed no evidence of harm to the fetus due to sincalide. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed (see WARNINGS).

Labor and Delivery

Sincalide should not be administered to pregnant women near term because of its effect on smooth muscle; the possibility of inducing labor prematurely exists. The effects of sincalide on labor, delivery and lactation in animals has not been determined (see WARNINGS).

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when sincalide is administered to a nursing woman.

Pediatric Use

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

Reactions to sincalide are generally mild and of short duration. The most frequent adverse reactions were abdominal discomfort or pain, and nausea; rapid intravenous injection of 0.04 mcg sincalide per kg expectably causes transient abdominal cramping. These phenomena are usually manifestations of the physiologic action of the drug, including delayed gastric emptying and increased intestinal motility. These reactions occurred in approximately 20 percent of patients; they are not to be construed as necessarily indicating an abnormality of the biliary tract unless there is other clinical or radiologic evidence of disease.

The incidence of other adverse reactions, including vomiting, flushing, sweating, rash, hypotension, hypertension, shortness of breath, urge to defecate, headache, diarrhea, sneezing, and numbness was less than 1 percent; dizziness was reported in approximately 2 percent of patients. These manifestations are usually lessened by slower injection rate.

OVERDOSAGE

Although no overdosage reports have been received, gastrointestinal symptoms (abdominal cramps, nausea, vomiting and diarrhea) would be expected. Hypotension with dizziness or fainting might also occur. Overdosage symptoms should be treated symptomatically and should be of short duration. Starting with single bolus i.v. injection comparable to the human dose of 0.4 mg/kg, sincalide caused hypotension and bradycardia in dogs. Higher doses injected once or repeatedly in dogs caused syncope and ECG changes in addition. These effects were attributed to sincalide-induced vagal stimulation in that all were prevented by pretreatment with atropine or bilateral vagotomy.

DOSAGE AND ADMINISTRATION

Reconstitution and Storage

Sincalide for injection may be stored at room temperature prior to reconstitution.

To reconstitute, aseptically add 5 mL of Sterile Water for Injection USP to the vial; any additional dilution should be made with Sodium Chloride Injection USP, 0.9%. The solution may be kept at room temperature and should be used within 24 hours of reconstitution, after which time any unused portion should be discarded.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

For prompt contraction of the gallbladder, a dose of 0.02 mcg sincalide per kg (1.4 mcg/70 kg) is injected intravenously over a 30- to 60-second interval; if satisfactory contraction of the gallbladder does not occur in 15 minutes, a second dose, 0.04 mcg sincalide per kg, may be administered. To reduce the intestinal side effects (see ADVERSE REACTIONS), an intravenous infusion may be prepared at a dose of 0.12 mcg/kg in 100 mL of Sodium Chloride Injection USP and given at a rate of 2 mL per minute; alternatively, an intramuscular dose of 0.1 mcg/kg may be given. When Kinevac (Sincalide for injection) is used in cholecystography, roentgenograms are usually taken at five-minute intervals after the injection. For visualization of the cystic duct, it may be necessary to take roentgenograms at one-minute intervals during the first five minutes after the injection.

For the Secretin-Kinevac test of pancreatic function, the patient receives a dose of 0.25 units secretin per kg by intravenous infusion over a 60-minute period. Thirty minutes after the initiation of the secretin infusion, a separate IV infusion of Kinevac at a total dose of 0.02 mcg per kg is administered over a 30-minute interval. For example, the total dose for a 70 kg patient is 1.4 mcg of sincalide; therefore, dilute 1.4 mL of reconstituted Kinevac solution to 30 mL with Sodium Chloride Injection USP and administer at a rate of 1 mL per minute.

To accelerate the transit time of a barium meal through the small bowel, administer Kinevac after the barium meal is beyond the proximal jejunum. (Sincalide, like cholecystokinin, may cause pyloric contraction.) The recommended dose is 0.04 mcg sincalide per kg (2.8 mcg/70 kg) injected intravenously over a 30- to 60-second interval; if satisfactory transit of the barium meal has not occurred in 30 minutes, a second dose of 0.04 mcg sincalide per kg may be administered. For reduction of side effects, a 30-minute IV infusion of sincalide [0.12 mcg per kg (8.4 mcg/70 kg) diluted to approximately 100 mL with Sodium Chloride Injection USP] may be administered.

HOW SUPPLIED

Kinevac (Sincalide for Injection) is supplied in packages of 10 vials containing 5 mcg of sincalide per vial (NDC 0270-0556-15).

Storage

Store at room temperature, 15°-30° C (59°-86° F).

Manufactured for
Bracco Diagnostics Inc.
Princeton, N.J. 08543
by E.R. Squibb & Sons Inc. New Brunswick, N.J. 08903

J3-398J Printed in USA Revised November-1994 J3-398J

KG	CJ	Wang and Hans[illegible]arenteral Formulations of Proteins and Peptides: [illegible]ility and Stabilizers," Journal of Parenteral Science & Technology, Vol. 42, Supplement 1988, pp. S3-S25
	CK	Carpenter et al., "Freezing- and Drying-Induced Perturbations of Protein Structure and Mechanisms of Protein Protection by Stabilizing Additives," Drugs and the Pharmaceutical Sciences, Vol. 96, 1999, pp. 123-160
	CL	Pikal, "Mechanisms of Protein Stabilization During Freeze-Drying and Storage: The Relative Importance of Thermodynamic Stabilization and Glassy State Relaxation Dynamics," Drugs and the Pharmaceutical Sciences, Vol. 96, 1999, pp. 161-197
	CM	Shah et al., "The Effects of Various Excipients on the Unfolding of Basic Fibroblast Growth Factor," PDA Journal of Pharmaceutical Science & Technology, Vol. 52, No. 5, September-October 1998, pp. 209-214
	CN	Powell et al., "Compendium of Excipients for Parenteral Formulations," PDA Journal of Pharmaceutical Science & Technology, Vol. 52, No. 5, September-October 1998, pp. 238-311
	CO	Zeissman, "Cholecystokinin Cholescintigraphy: Victim of Its Own Success?" Journal of Nuclear Medicine, Vol. 40, No. 12, December 1999, pp. 2038-2042
	CP	Krishnamurthy and Krishnamurthy, "Gallbladder Ejection Fraction: A Decade of Progress and Future Promise," Journal of Nuclear Medicine, Vol. 32, No. 4, April 1992, pp. 542-544
	CQ	Krishnamurthy et al., "Quantitative Biliary Dynamics: Introduction of a New Noninvasive Scintigraphic Technique," Journal of Nuclear Medicine, Vol. 24, No. 3, 1983, pp. 217-223
	CR	Mesgarzadeh et al., "Filling, Postcholecystokinin Emptying, and Refilling of Normal Gallbladder: Effects of Two Different Doses of CCK on Refilling: Concise Communication," Journal of Nuclear Medicine, Vol. 24, No. 8, 1983, pp. 666-671
	CS	Krishnamurthy et al., "The Gallbladder Emptying Response to Sequential Exogenous and Endogenous Cholecystokinin," Nuclear Medicine Communications, Vol. 5, 1984, pp. 27-33
	CT	Krishnamurthy et al., "Detection, Localization, and Quantitation of Degree of Common Bile Duct Obstruction by Scintigraphy," Journal of Nuclear Medicine, Vol. 26, No. 7, July 1985, pp. 726-735
	CU	Fink-Bennett et al., "Cholecystokinin Cholescintigraphic Findings in the Cystic Duct Syndrome," Journal of Nuclear Medicine, Vol. 26, No. 10, October 1985, pp. 1123-1128
	CV	Fink-Bennett, "The Role of Cholecystogogues in the Evaluation of Biliary Tract Disorders," Nuclear Medicine Annual 1985, Lenny Freeman and Heidi Weissman, eds., New York, Raven Press, 1985, pp. 107-132
	CW	Newman et al., "A Simple Technique for Quantitative Cholecystokinin – HIDA Scanning," The British Journal of Radiology, Vol. 56, July 1983, pp. 500-502
	CX	Pickleman et al., "The Role of Sincalide Cholescintigraphy in the Evaluation of Patients with Acalculus Gallbaldder Disease," Archives of Surgery, Vol. 120, June 1985, pp. 693-697
	CY	Zeissman et al., "Calculation of a Gallbaldder Ejection Fraction: Advantage of Continuous Sincalide Infusion Over the Three-Minute Infusion Method," Journal of Nuclear Medicine, Vol. 33, No. 4, April 1992, pp. 537-541
KG	CZ	Balon et al., Society of Nuclear Medicine Procedure Guideline for Hepatobiliary Scintigraphy
	CAA	
	CBB	
	CCC	
	CDD	
	CEE	
	CFF	
	CGG	
	CHH	

EXAMINER	DATE CONSIDERED 7.21.04

* **EXAMINER:** Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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NOTICE OF ALLOWANCE AND FEE(S) DUE

35743 7590 07/27/2004

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
GEORGE, KONATA M	
ART UNIT	PAPER NUMBER
1616	

DATE MAILED: 07/27/2004

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

TITLE OF INVENTION: SINCALIDE FORMULATIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	10/27/2004

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE REFLECTS A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE APPLIED IN THIS APPLICATION. THE PTOL-85B (OR AN EQUIVALENT) MUST BE RETURNED WITHIN THIS PERIOD EVEN IF NO FEE IS DUE OR THE APPLICATION WILL BE REGARDED AS ABANDONED.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 07/04) Approved for use through 04/30/2007.

PART B - FEE(S) TRANSMITTAL

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Alexandria, Virginia 22313-1450

or Fax (703) 746-4000

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CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

35743 7590 07/27/2004

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NEW YORK, NY 10022

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

TITLE OF INVENTION: SINCALIDE FORMULATIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	10/27/2004

EXAMINER	ART UNIT	CLASS-SUBCLASS
GEORGE, KONATA M	1616	424-489000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively, 1_____

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2_____ 3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent); ❑ individual ❑ corporation or other private group entity ❑ government

4a. The following fee(s) are enclosed:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s):

❑ A check in the amount of the fee(s) is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is not claiming SMALL ENTITY status. See, e.g., 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

(Authorized Signature) (Date)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

35743 7590 07/27/2004

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INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

EXAMINER	
GEORGE, KONATA M	

ART UNIT	PAPER NUMBER
1616	

DATE MAILED: 07/27/2004

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (703) 305-1383. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 07/04) Approved for use through 04/30/2007.

Notice of Allowability	Application No.	Applicant(s)	
	10/222,540	METCALFE ET AL.	
	Examiner	Art Unit	
	Konata M. George	1616	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *RCE filed December 22, 2003*.

2. ☒ The allowed claim(s) is/are *1-108*.

3. ☒ The drawings filed on *August 16, 2002* are accepted by the Examiner.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)
1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date 10
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.
7. ☐ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

DETAILED ACTION

Claims 1-108 are pending in this application.

Request for Continued Examination (RCE)

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after allowance or after an Office action under *Ex Parte Quayle*, 25 USPQ 74, 453 O.G. 213 (Comm'r Pat. 1935). Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on December 22, 2003 has been entered.

Information Disclosure Statement

2. The information disclosure statement (IDS) submitted on December 22, 2003 was noted and the submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the examiner has considered the information disclosure statement.

Allowable Subject Matter

3. Claims 1-108 are allowed for the reasons stated in the office action dated October 3, 2003. The submission of the IDS does not contain any references that would constitute prior art.

Telephone Inquiries

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Konata M. George, whose telephone number is (571) 272-0613. The examiner can normally be reached from 8AM to 5:30PM Monday to Thursday, and on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz, can be reached at (571) 272-0887. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 872-9306 for regular communications and for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

Konata M. George

Gary S. Kunz

GARY KUNZ
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Customer No. 35743

Docket No. 57637/1160

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	Metcalfe, Edmund et al.	Confirmation No. 6555
Serial No.	10/222,540	
Filed	August 16, 2002	
For	**SINCALIDE FORMULATIONS**	
Group Art Unit	1616	
Examiner	George, Konata M.	

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on **August 19, 2004.**

Signature: C-Caggiano

Carrie L. Caggiano

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ISSUE FEE TRANSMITTAL

Sir:

In payment of the Issue Fee and Publication Fee mailed in the present application by the Notice of Allowance dated July 27, 2004 Applicants respectfully submit the following documents herewith:

- Completed Part B-Fee(s) Transmittal (PTOL-85B);
- Certificate of First Class Mailing under 37 C.F.R.. § 1.8 dated August 19, 2004
- Kramer Levin Naftalis & Frankel LLP check in the amount of $1660.00
- Return Receipt Postcard.

The Director is hereby authorized to charge any additional fee(s) or credit any overpayment to Deposit Account No. 50-0540.

Respectfully submitted,

Dated: August 19, 2004

Donald L. Rhoads, Reg. No. 34,705
Albert B. Chen, Reg. No. 41,667
KRAMER LEVIN NAFTALIS & FRANKEL LLP
919 Third Avenue
New York, New York 10022
Tel: (212) 715-9100
Fax: (212) 715-8000

KL3:2309851.2

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (703) 746-4000

AUG 23 2004

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

35743 7590 07/27/2004

KRAMER LEVIN NAFTALIS & FRANKEL LLP
INTELLECTUAL PROPERTY DEPARTMENT
919 THIRD AVENUE
NEW YORK, NY 10022

08/24/2004 MMEKONE1 00000001 10222540

01 FC:1501 1330.00 OP
02 FC:1504 300.00 OP

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

Carrie L. Caggiano	(Depositor's name)
	(Signature)
	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/222,540	08/16/2002	Edmund C. Metcalfe	50203/017001	6555

TITLE OF INVENTION: SINCALIDE FORMULATIONS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	10/27/2004

EXAMINER	ART UNIT	CLASS-SUBCLASS
GEORGE, KONATA M	1616	424-489000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 ~~Kramer, Levin, Naftalis~~
2 & Frankel LLP
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Bracco International B.V.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Amsterdam, The Netherlands

Please check the appropriate assignee category or categories (will not be printed on the patent); ☐ individual ☒ corporation or other private group entity ☐ government

4a. The following fee(s) are enclosed:
☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☒ Advance Order - # of Copies 10

4b. Payment of Fee(s):
☒ A check in the amount of the fee(s) is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number 50-0540 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is not claiming SMALL ENTITY status. See, e.g., 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

(Authorized Signature) (Date)
Reg No. 41,667 8/19/04

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE(S)