

PHARMACEUTICAL COMPOSITION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a PCT International Application claiming benefit to U.S. Provisional Application Serial No. 63/477,264, filed December 27, 2022, and U.S. Provisional Application Serial No. 63/295,076, filed December 30, 2021, each of which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[0002] Described herein are compositions that comprise Bumetanide Dibenzyamide for treating selected conditions of the central and peripheral nervous systems employing non-synaptic mechanisms. More specifically, the present disclosure relates to methods and compositions for treating neurological disorders by administering agents that disrupt hypersynchronized neuronal activity without diminishing neuronal excitability. These compositions are useful for seizure disorders including epilepsy and related indications.

BACKGROUND

[0003] Epilepsy is characterized by abnormal discharges of cerebral neurons and is typically manifested as various types of seizures. Many anti-convulsants originally developed for the treatment of epilepsy and other seizure disorders have also found application in the treatment of non-epileptic conditions, including neuropathic pain, mood disorders (such as bipolar affective disorder), and schizophrenia (for a review of the use of anti-epileptic drugs in the treatment of non-epileptic conditions, see Rogawski and Loscher, *Nat. Medicine*, 10:685- 692, 2004). It has thus been suggested that epilepsy, neuropathic pain and affective disorders have a common pathophysiological mechanism (Rogawski & Loscher, *ibid*; Ruscheweyh & Sandkuhler, *Pain* 105:327-338, 2003), namely a pathological increase in neuronal excitability, with a corresponding inappropriately high frequency of spontaneous firing of neurons. However, only some, and not all, antiepileptic drugs are effective in treating neuropathic pain, and furthermore such antiepileptic drugs are

only effective in certain subsets of patients with neuropathic pain (McCleane, *Expert. Opin. Pharmacother.* 5:1299-1312, 2004).

[0004] Epileptiform activity is identified with spontaneously occurring synchronized discharges of neuronal populations that can be measured using electrophysiological techniques. This synchronized activity, which distinguishes epileptiform from non-epileptiform activity, is referred to as "hypersynchronization" because it describes the state in which individual neurons become increasingly likely to discharge in a time-locked manner with one another. Hypersynchronized activity is typically induced in experimental models of epilepsy either by increasing excitatory or by decreasing inhibitory synaptic currents. It was therefore assumed that hyperexcitability per se was the defining feature involved in the generation and maintenance of epileptiform activity. Similarly, neuropathic pain was believed to involve conversion of neurons involved in pain transmission from a state of normal sensitivity to one of hypersensitivity (Costigan & Woolf, *Jnl. Pain* 1: 35-44, 2000). The focus on developing treatments for both epilepsy and neuropathic pain has thus been on suppressing neuronal hyperexcitability by either: (a) suppressing action potential generation; (b) increasing inhibitory synaptic transmission, or (c) decreasing excitatory synaptic transmission.

[0005] Most agents currently used for treatment target synaptic activity in excitatory pathways by, for example, modulating the release or activity of excitatory neurotransmitters, potentiating inhibitory pathways, blocking ion channels involved in impulse generation, and/or acting as membrane stabilizers. Conventional agents and therapeutic approaches for the treatment of epilepsy and neuropsychiatric disorders thus reduce neuronal excitability and inhibit synaptic firing. One serious drawback of these therapies is that they are nonselective and exert their actions on both normal and abnormal neuronal populations. This leads to negative and unintended side effects, which may affect normal CNS functions, such as cognition, learning and memory, and produce adverse physiological and psychological effects in the treated patient. Common side effects include over-sedation, dizziness, loss of memory and liver damage. However, it has been shown that hypersynchronous epileptiform activity can be dissociated from hyperexcitability and that the cation chloride cotransport inhibitor furosemide can reversibly block synchronized

discharges without reducing hyperexcited synaptic responses (Hochman et al. *Science* 270:99-102, 1995).

[0006] The cation-chloride co-transporters (CCCs) are important regulators of neuronal chloride concentration that are believed to influence cell-to-cell communication, and various aspects of neuronal development, plasticity, and trauma. The CCC gene family consists of three broad groups: $\text{Na}^+\text{-Cl}^-$ co-transporters (NCCs), $\text{K}^+\text{-Cl}^-$ co-transporters (KCCs) and $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ co-transporters (NKCCs). Na-K-Cl co-transport in all cell and tissues is inhibited by loop diuretics, including furosemide, bumetanide, and benzmetanide. Espinosa et al. and Ahmad et al. have previously suggested that furosemide might be useful in the treatment of certain types of epilepsy (*Medicina Espanola* 61:280-281, 1969; and *Brit. J. Clin. Pharmacol.* 3:621-625, 1976). Bumetanide is potentially a more potent drug for treating epilepsy, but it also has a more pronounced diuretic effect. There is therefore a continuing need for methods and compositions for treating neuronal disorders that are not diuretic and that disrupt hypersynchronized neuronal activity without diminishing the neuronal excitability and spontaneous synchronization required for normal functioning of the peripheral and central nervous systems.

SUMMARY

[0007] One embodiment of the present disclosure includes a pharmaceutical composition comprising Bumetanide Dibenzyamide and one or more solubilizers.

[0008] In one aspect, the composition comprises about 2.5 mg/mL to about 42 mg/mL of Bumetanide Dibenzyamide. In one aspect, the composition comprises about 0.25%w/w to about 15%w/w of Bumetanide Dibenzyamide. In one aspect, the composition comprises about 0.1%w/w to about 99.75%w/w of one or more solubilizers. In one aspect, the one or more solubilizers comprise short chain triglycerides, long chain triglycerides, or combinations thereof. In one aspect, the one or more solubilizers comprise Polyoxyl 35 Castor Oil, Glyceryl Monolinoleate, or any combination thereof. In one aspect, the one or more solubilizers comprise Caprylocaproyl Polyoxylglycerides, Phosphatidylcholine, Caprylic/Capric Triglyceride, Lauroyl Polyoxyl-32 Glycerides, Sorbitan Ester, or any combination thereof. In one aspect, the one or more solubilizers comprise Ethanol,

Propylene Glycol, Polyethylene Glycol 600, Polyethylene Glycol 3350, Oleyl Alcohol, or any combination thereof. In one aspect, the one or more solubilizers comprise PEG-400, Vitamin E TPGS, or any combination thereof. In one aspect, the one or more solubilizers comprises soybean oil. In one aspect, the one or more solubilizers comprises water. In one aspect, the one or more solubilizers comprise Polyvinylpyrrolidone (K30), Poloxamer 407 (P407), Sodium Carboxymethyl cellulose (CMC), or any combination thereof. In one aspect, the one or more solubilizers comprises at least one super disintegrant. In one aspect, the one or more solubilizers comprises at least one wetting agents. In one aspect, the one or more solubilizers comprises at least one surfactants. In one aspect, the one or more solubilizers comprise Ceolus KG (microcrystalline cellulose), Mannogem EZ (spray dried mannitol), Polyplasdone XL (super disintegrate), Poloxamer 407, Lauroyl Polyoxyl-32 Glycerides, Sorbitan Ester, Neusilin US2 (magnesium aluminometasilicate), Citric acid Monohydrate, Cabosil M5P (fumed silica), Magnesium Stearate, or any combination thereof.

[0009] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.79 %w/w of Bumetanide Dibenzylamide, about 33.48 %w/w of Polyoxyl 35 Castor oil, about 32.37 %w/w of Glyceryl Monolinoleate, and about 32.37 %w/w of Soybean oil.

[0010] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.76 %w/w of Bumetanide Dibenzylamide, about 32.93 %w/w of Polyoxyl 35 Castor oil, about 31.83 %w/w of Glyceryl Monolinoleate, about 31.83 %w/w of Soybean oil, and about 10.37 %w/w of Ethanol.

[0011] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.59 %w/w of Bumetanide Dibenzylamide, about 22 %w/w of Phosphatidylcholine, about 70 %w/w of Caprylocaproyl Polyoxylglycerides, and about 6.41 %w/w of Caprylic/Capric Triglyceride.

[0012] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.98 %w/w of Bumetanide Dibenzylamide, about 19.39 %w/w of Lauroyl

Plyoxyl-32 Glycerides, about 37.62 %w/w of Sorbitan Ester, and about 41.01 %w/w of Soybean oil.

[0013] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.98 %w/w of Bumetanide Dibenzylamide, about 39.72 %w/w of Caprylocaproyl Polyoxylglycerides, about 25.94 %w/w of Sorbitan Ester, and about 49.78 %w/w of Soybean oil.

[0014] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.73 %w/w of Bumetanide Dibenzylamide, about 32.40 %w/w of Polyoxyl 35 Castor oil, about 31.32 %w/w of Glyceryl Monolinoleate, about 31.32 %w/w of Soybean oil, and about 3.24 %w/w of Ethanol.

[0015] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 1.7 %w/w of Bumetanide Dibenzylamide, about 31.91 %w/w of Polyoxyl 35 Castor oil, about 30.85 %w/w of Glyceryl Monolinoleate, about 30.85 %w/w of Soybean oil, and about 4.68 %w/w of Ethanol.

[0016] One aspect of the present disclosure includes a pharmaceutical composition comprising about 10 %w/w to about 100 %w/w of Caprylocaproyl Polyoxylglycerides. One aspect of the present disclosure includes a pharmaceutical composition comprising about 12 %w/w to about 20 %w/w of Propylene Glycol. One aspect of the present disclosure includes a pharmaceutical composition comprising about 57 %w/w to about 80 %w/w of PEG 400. One aspect of the present disclosure includes a pharmaceutical composition comprising about 1% Vitamin E TPGS.

[0017] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 2.73 %w/w of Bumetanide Dibenzylamide, about 10 % w/w of Caprylocaproyl Polyoxylglycerides, about 12.05 %w/w of Propylene Glycol, about 67.22 %w/w of Polyethylene Glycol 400, and about 8 %w/w of Water.

[0018] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 2.82 %w/w of Bumetanide Dibenzylamide, about 4.65 % w/w of

Caprylocaproyl Polyoxylglycerides, about 13.01 %w/w of Propylene Glycol, about 67.97 %w/w of Polyethylene Glycol 400, about 0.92 %w/w Polyvinylpyrrolidone (K30), and about 10.62 %w/w of Water.

[0019] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 0.6 %w/w of Bumetanide Dibenzylamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 53.84 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), about 0.41 %w/w Sodium CMC, and about 20 %w/w of water.

[0020] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 0.6 %w/w of Bumetanide Dibenzylamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 66.75 %w/w of Polyethylene Glycol 400, about 3. %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), and about 7.5 %w/w of Polyethylene Glycol 3350.

[0021] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 15 %w of Bumetanide Dibenzylamide, about 20 %w of Microcrystalline Cellulose, about 51 %w of Spray Dried Mannitol, about 7 %w of Polyplasdone XL (super disintegrant), about 3% w of Poloxamer 407, about 1.5 %w of Citric Acid Monohydrate, about 1.0 %w of Cabosil M5P (fumed silica), and about 1.5 %w of Magnesium Stearate.

[0022] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 7.4 %w of Bumetanide Dibenzylamide, about 9.9 %w of Lauroyl Polyoxyl-32 Glycerides, about 9.9 %w of Sorbitan Ester, about 54.3 %w of Polyplasdone XL (super disintegrate), about 0.5% w of Poloxamer 407, about 0.7 %w of Citric Acid Monohydrate, about 2.0 %w of Cabosil M5P (fumed silica), about 14.8 %w of Neusilin US2 (magnesium aluminometasilicate), and about 0.5 %w of Magnesium Stearate.

[0023] One embodiment of the present disclosure includes a pharmaceutical composition comprising a vehicle composition comprising 55 % Polyethylene Glycol 400 (PEG-400) in water, or 41 % PEG-400, 12 % Ethanol, 47 % water or 45 % PEG-400, 10 % DMSO, 45 %

water, or 31 % PEG-400, 31 % Tetraglycol, 15 % Caprylocaproyl Polyoxylglycerides, 23 % water, or 30 % PEG-400, 10 % N-Methyl Pyrrolidone, 10 % DMSO, 50 % water, or 25 % PEG-400, 10 % DMSO, 20 % Tetraglycol, 45 % water, or 20 % Ethanol in water, or 20% Hydroxypropyl- β -Cyclodextrin in water, or 11% DMSO, 22% PEG-400, 22% Tetraglycol, 44% water, or 44% PEG-400, 0.4% Poloxamer-188, 22% N-Methyl Pyrrolidone, 33% water, or 20% PEG-400, 15% Hydroxypropyl- β -Cyclodextrin in water, or 40% PEG-400, 20% Propylene Glycol, 5% Ethanol and 35%, water.

[0024] One embodiment of the present disclosure includes a pharmaceutical composition comprising about 28 g of Bumetanide Dibenzylamide, and about 3 ml of a solvent, wherein 50 g of solvent comprises about 5g of Caprylocaproyl Polyoxylglycerides, about 10g of Propylene Glycol, about 28.5g of PEG-400, about 0.5g of Vitamin E TPGS, about 1g of Ethanol, and about 4.5g of water.

[0025] One embodiment of the present disclosure includes a method for treating a patient in need thereof comprising: administering a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers.

[0026] In an alternative embodiment, the present disclosure includes a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers for use in mecidine.

[0027] In a further alternative embodiment, there is provided use of a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers in the manufacture of a medicament. In a further embodiment, a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers may used of the manufacture of a medicament for the therapeutic and/or prophylactic treatment of seizure, epilepsy, and/or other indications such as neuropathic pain. In some embodiments, a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers may used of the manufacture of a medicament for the therapeutic and/or prophylactic treatment of epilepsy and/or neurological syndromes that are specific to children. In some embodiments, a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers may used of the manufacture of a medicament for

the therapeutic and/or prophylactic treatment of one or more indications listed in Figure 18 (Fisher et al.) and for treating whatever terms might replace the old and current terms describing the indications listed in Figure 18 in the future. In some embodiments, a pharmaceutical composition comprising Bumetanide Dibenzyamide and one or more solubilizers may be used in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of migraines or tinnitus. In some embodiments, a pharmaceutical composition comprising Bumetanide Dibenzyamide and one or more solubilizers may be used in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of depression and/or anxiety. In some embodiments, a pharmaceutical composition comprising Bumetanide Dibenzyamide and one or more solubilizers may be used in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of psychoneurotic disorders. In some embodiments, a pharmaceutical composition comprising Bumetanide Dibenzyamide and one or more solubilizers may be used in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of neurodegenerative disorders including but not limited to Alzheimer's disease, amyotrophic lateral sclerosis, Friedrich ataxia, Huntington's Disease, Lewy Body disease, Parkinson's disease, or spinal muscular atrophy. In one aspect, the pharmaceutical composition comprises about 2.5 mg/mL to about 42 mg/mL of Bumetanide Dibenzyamide. In one aspect, the pharmaceutical composition comprises about 0.25%w/w to about 15%w/w of Bumetanide Dibenzyamide. In one aspect, the one or more solubilizers comprise short chain triglycerides, long chain triglycerides, or combinations thereof. In one aspect, the one or more solubilizers comprise polyoxyl 35 castor oil, Glyceryl Monolinoleate, or any combination thereof. In one aspect, the one or more solubilizers comprise Caprylocaproyl Polyoxylglycerides, Phosphatidylcholine, Caprylic/Capric Triglyceride, Lauroyl Polyoxyl-32 Glycerides, Sorbitan Ester or any combination thereof. In one aspect, the one or more solubilizers comprise Ethanol, Propylene Glycol, Polyethylene Glycol 600, Polyethylene Glycol 3350, Oleyl Alcohol, or any combination thereof. In one aspect, the one or more solubilizers comprise PEG-400, Vitamin E TPGS, or any combination thereof. In one aspect, the one or more solubilizers comprise Soybean oil. In one aspect, the one or more solubilizers comprise water. In one aspect, the one or more solubilizers comprise Polyvinylpyrrolidone (K30), Poloxamer 407 (P407), Sodium Carboxymethyl cellulose (CMC), or any combination thereof. In one

aspect, the one or more solubilizers comprises at least one super disintegrant. In one aspect, the one or more solubilizers comprises at least one wetting agent. In one aspect, the one or more solubilizers comprises at least one surfactant. In one aspect, the one or more solubilizers comprise Ceolus KG (microcrystalline cellulose), Mannogem EZ (spray dried mannitol), Polyplasdone XL (super disintegrate), Poloxamer 407, lauroyl polyoxyl-32 glycerides, sorbitan ester, Neusilin US2 (magnesium aluminometasilicate), Citric acid Monohydrate, Cabosil M5P (fumed silica), Magnesium Stearate or any combination thereof. In one aspect, the pharmaceutical composition comprising about 1.79 %w/w of Bumetanide Dibenzyamide, about 33.48 %w/w of Polyoxyl 35 Castor oil, about 32.37 %w/w of Glyceryl Monolinoleate, and about 32.37 %w/w of Soybean oil. In one aspect, the pharmaceutical composition comprising about 1.76 %w/w of Bumetanide Dibenzyamide, about 32.93 %w/w of Polyoxyl 35 Castor oil, about 31.83 %w/w of Glyceryl Monolinoleate, about 31.83 %w/w of Soybean oil, and about 10.37 %w/w of Ethanol. In one aspect, the pharmaceutical composition comprising about 1.59 %w/w of Bumetanide Dibenzyamide, about 22 %w/w of Phosphatidylcholine, about 70 %w/w of Caprylocaproyl Polyoxylglycerides, and about 6.41 %w/w of Caprylic/Capric Triglyceride. In one aspect, the pharmaceutical composition comprising about 1.98 %w/w of Bumetanide Dibenzyamide, about 19.39 %w/w of Lauroyl Polyoxyl-32 Glycerides, about 37.62 %w/w of Sorbitan Ester, and about 41.01 %w/w of Soybean oil. In one aspect, the pharmaceutical composition comprising about 1.98 %w/w of Bumetanide Dibenzyamide, about 39.72 %w/w of Caprylocaproyl Polyoxylglycerides, about 25.94 %w/w of Sorbitan Ester, and about 49.78 %w/w of Soybean oil. In one aspect, the pharmaceutical composition comprising about 1.73 %w/w of Bumetanide Dibenzyamide, about 32.40 %w/w of Polyoxyl 35 Castor oil, about 31.32 %w/w of Glyceryl Monolinoleate, about 31.32 %w/w of Soybean oil, and about 3.24 %w/w of Ethanol. In one aspect, the pharmaceutical composition comprising about 1.7 %w/w of Bumetanide Dibenzyamide, about 31.91 %w/w of Polyoxyl 35 Castor oil, about 30.85 %w/w of Glyceryl Monolinoleate, about 30.85 %w/w of Soybean oil, and about 4.68 %w/w of Ethanol. In one aspect, the pharmaceutical composition comprising about 10 %w/w to about 100 %w/w of Caprylocaproyl Polyoxylglycerides. In one aspect, the pharmaceutical composition comprising about 12 %w/w to about 20 %w/w of Propylene Glycol. In one aspect, the pharmaceutical

composition comprising about 57 %w/w to about 80 %w/w of PEG 400. In one aspect, the pharmaceutical composition comprising about 1% Vitamin E TPGS. In one aspect, the pharmaceutical composition comprising about 2.73 %w/w of Bumetanide Dibenzylamide, about 10 % w/w of Caprylocaproyl Polyoxylglycerides, about 12.05 %w/w of Propylene Glycol, about 67.22 %w/w of Polyethylene Glycol 400, and about 8 %w/w of Water. In one aspect, the pharmaceutical composition comprising about 2.82 %w/w of Bumetanide Dibenzylamide, about 4.65 % w/w of Caprylocaproyl Polyoxylglycerides, about 13.01 %w/w of Propylene Glycol, about 67.97 %w/w of Polyethylene Glycol 400, about 0.92 %w/w Polyvinylpyrrolidone (K30), and about 10.62 %w/w of Water. In one aspect, the pharmaceutical composition comprising about 0.6 %w/w of Bumetanide Dibenzylamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 53.84 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), about 0.41 %w/w Sodium CMC, and about 20 %w/w of water. In one aspect, the pharmaceutical composition comprising about 0.6 %w/w of Bumetanide Dibenzylamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 66.75 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), and about 7.5 %w/w of Polyethylene Glycol 3350. In one aspect, the pharmaceutical composition comprising about 15 %w of Bumetanide Dibenzylamide, about 20 %w of Microcrystalline Cellulose, about 51 %w of Spray Dried Mannitol, about 7 %w of Polyplasdone XL (super disintegrate), about 3% w of Poloxamer 407, about 1.5 %w of Citric Acid Monohydrate, about 1.0 %w of Cabosil M5P (fumed silica), and about 1.5 %w of Magnesium Stearate. In one aspect, the pharmaceutical composition comprising about 7.4 %w of Bumetanide Dibenzylamide, about 9.9 %w of Lauroyl Polyoxyl-32 Glycerides, about 9.9 %w of Sorbitan Ester, about 54.3 %w of Polyplasdone XL (super disintegrate), about 0.5% w of Poloxamer 407, about 0.7 %w of Citric Acid Monohydrate, about 2.0 %w of Cabosil M5P (fumed silica), about 14.8 %w of Neusilin US2 (magnesium aluminometasilicate), and about 0.5 %w of Magnesium Stearate. In one aspect, the pharmaceutical composition comprising a vehicle composition comprising 55 Polyethylene Glycol 400 (PEG-400) in water, or 41 % PEG-400, 12 % ethanol, 47 % water or 45 % PEG-400, 10 % DMSO, 45 % water, or 31 % PEG-400, 31 % Tetraglycol, 15 %

Caprylocaproyl Polyoxylglycerides, 23 % water, or 30 % PEG-400, 10 % N-Methyl Pyrrolidone, 10 % DMSO, 50 % water, or 25 % PEG-400, 10 % DMSO, 20 % Tetraglycol, 45 % water, or 20 % Ethanol in water, or 20% Hydroxypropyl- β -Cyclodextrin in water, or 11% DMSO, 22% PEG-400, 22% Tetraglycol, 44 % water, or 44 % PEG-400, 0.4 % Poloxamer-188, 22 % N-Methyl pyrrolidone, 33 % water, or 20 % PEG-400, 15 % Hydroxypropyl- β -Cyclodextrin in water, or 40 % PEG-400, 20 % Propylene Glycol, 5% Ethanol, and 35% water. In one aspect, the pharmaceutical composition comprising about 28 g of Bumetanide Dibenzyamide, and about 3 ml of a solvent, wherein 50 g of solvent comprises about 5g of Caprylocaproyl Polyoxylglycerides, about 10g of Propylene Glycol, about 28.5g of PEG-400, about 0.5g of Vitamin E TPGS, about 1g of Ethanol, and about 4.5g of water. In one aspect, the pharmaceutical composition is an oral capsule. In one aspect, the pharmaceutical composition is a nasal solution. In one aspect, the pharmaceutical composition is a rectal gel. In one aspect, the pharmaceutical composition is a rectal paste. In one aspect, the pharmaceutical composition is a sublingual tablet. In one aspect, the pharmaceutical composition is an injectable composition. In one aspect, the dosing of the patient with the pharmaceutical composition is done orally. In one aspect, the dosing of the patient with the pharmaceutical composition is done intranasally. In one aspect, the dosing of the patient with the pharmaceutical composition is done rectally. In one aspect, the dosing of the patient with the pharmaceutical composition is done sublingually. In one aspect, the dosing of the patient with the pharmaceutical composition is done subcutaneously. In one aspect, the dosing of the patient with the pharmaceutical composition is done intramuscularly.

[0028] One embodiment of the present disclosure includes a pharmaceutical composition comprising Bumetanide Dibenzyamide and one or more organic anion transport (OAT) inhibitors. The role of active transport in tissue distribution of bumetanide has been extensively studied for the kidney and the liver but not the BBB. In mice and humans, OATs are thought to mediate the renal transport of bumetanide. These transporters maybe essentially related to the transport of bumetanide on the luminal and basolateral membrane site of the kidney proximal tubular cells. Bumetanide seems not to be transported by OATPs in kidney or liver, although only few members of this transporter family have been studied in this respect. Probenecid inhibits OATs which explains that it has been shown to reduce the plasma and renal clearance of

bumetanide in dogs. In line with these observations in dogs, probenecid markedly increased the plasma half-life of bumetanide in mice. However, it did not decrease the diuretic effect of bumetanide. Systemic administration of an organic anion transport inhibitor may have a beneficial effect on brain levels of bumetanide or products thereof. For background teaching, see Tollner et al., European Journal of Pharmacology, 746 (2015) 167-173, herein incorporated by reference with regard to such teaching.

[0029] In one aspect, the one or more OAT inhibitors are competitive antagonists. In one aspect, the Bumetanide Dibenzyamide and the one or more OAT inhibitors are formulated with different release profiles. In one aspect, the at least one OAT inhibitor is formulated to be released prior to release of the Bumetanide Dibenzyamide. In one aspect, the at least one OAT inhibitor is formulated to be released prior to the Bumetanide Dibenzyamide achieving maximum plasma concentration ($C_{\max}$) in a patient receiving the composition. In one aspect, wherein the one or more OAT inhibitor is selected from the group consisting of probenecid, aspirin, ibuprofen, acetylsalicylic acid, diclofenac, aspartame, and valproic acid.

[0030] One embodiment of the present disclosure includes a pharmaceutical composition comprising Bumetanide Dibenzyamide in a self-emulsifying drug delivery system (SEDDS). In one aspect, the SEDDS comprises an isotropic mixture of oils, solubilizers, surfactants, and co-solvents.

[0031] One embodiment of the present disclosure includes a pharmaceutical composition comprising Bumetanide Dibenzyamide in an oral lymphatic targeted formulation.

[0032] In one aspect, the composition comprises Bumetanide Dibenzyamide, Polyoxyl 35 Castor Oil (Kolliphor EL), Glyceryl Monolinoleate (Maisine CC), Soybean Oil, and Ethanol. In one aspect, the composition comprises about 1 to 2 w/w% Bumetanide Dibenzyamide, about 30 to 35 w/w% Polyoxyl 35 Castor Oil (Kolliphor EL), about 30 to 35 w/w% Glyceryl Monolinoleate (Maisine CC), about 30 to 35 w/w% Soybean Oil, and about 2.5 to 5 w/w% Ethanol. In one aspect, the composition comprises about 1.7 w/w% Bumetanide Dibenzyamide, about 32.4 w/w% Polyoxyl 35 Castor Oil (Kolliphor EL), about 31.3 w/w% Glyceryl Monolinoleate (Maisine CC), about 31.3 w/w% Soybean Oil, and about 3.2 w/w% Ethanol.

[0033] One embodiment of the present disclosure includes a pharmaceutical composition comprising a prodrug of Bumetanide and one or more oral lymphatic targeting excipients. A prodrug of Bumetanide may include any compound comprising Bumetanide including but not limited to amide prodrug forms, which have been demonstrated to offer one or more unexpected benefits over other prodrug forms.

[0034] In one aspect, the prodrug of Bumetanide is an amide prodrug. In one aspect, the prodrug of Bumetanide is one or more of Bumetanide Dibenzylamide, Bumetanide Diethylamide, and Bumetanide Morpholinoamide. In one aspect the composition comprises alkoxyated castor oil. In one aspect the composition comprises Polyoxyl 35 Castor Oil (Kolliphor EL). In one aspect the composition comprises one or more of a mono-, di-, and triglyceride. In one aspect the composition comprises Glyceryl Monolinoleate (Maisine CC). In one aspect the composition comprises a fixed oil. In one aspect, the composition comprises soybean oil. In one aspect the composition comprises a water soluble solvent. In one aspect, the composition comprises ethanol.

[0035] For the avoidance of doubt, the prodrugs of Bumetanide described herein (e.g. Bumetanide Dibenzylamide, Bumetanide Diethylamide, and Bumetanide Morpholinoamide), and compositions comprising prodrugs of Bumetanide, may be suitable and/or preferred treatment agents for use in the methods of the present disclosure (including all aspects and embodiments of those methods). The prodrugs of Bumetanide may be used to treat epilepsy, Alzheimer, and other diseases and indication comprising seizure.

[0036] In one aspect, the pharmaceutical composition comprises up to 17.5 %w of Bumetanide Dibenzylamide, about 20 %w solublizer, and about 15 %w absorbant.

[0037] Throughout the present disclosure, any description of a method may be interpreted to describe and support a commensurate use, manufacture for use, composition, composition for use, or other alternative description.

[0038] One or more embodiments or aspects may be incorporated in a different embodiment or aspect although not specifically described. That is, all embodiments and aspects can be combined in any way or combination.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] FIG. 1A shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following IN administration of 15 mg Bumetanide Dibenzylamide to male and female Dogs on a linear scale.

[0040] FIG. 1B shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following IN administration of 15 mg Bumetanide Dibenzylamide to male and female Dogs on a log-linear scale.

[0041] FIG. 2A shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following SL administration of 30 mg Bumetanide Dibenzylamide to male and female Dogs on a linear scale.

[0042] FIG. 2B shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following SL administration of 30 mg Bumetanide Dibenzylamide to male and female Dogs on a log-linear scale.

[0043] FIG. 3A shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following IM administration of 15 mg Bumetanide Dibenzylamide to male and female Dogs on a linear scale.

[0044] FIG. 3B shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following IM administration of 15 mg Bumetanide Dibenzylamide to male and female Dogs on a log-linear scale.

[0045] FIG. 4A shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following PO administration of 30 mg Bumetanide Dibenzylamide to male and female Dogs on a linear scale.

[0046] FIG. 4B shows the individual animal Bumetanide Dibenzylamide concentration-time profiles following PO administration of 30 mg Bumetanide Dibenzylamide to male and female Dogs on a log-linear scale.

[0047] FIG. 5A shows the individual animal Bumetanide Dibenzyamide concentration-time profiles following SC administration of 15 mg Bumetanide Dibenzyamide to male and female Dogs on a linear scale.

[0048] FIG. 5B shows the individual animal Bumetanide Dibenzyamide concentration-time profiles following SC administration of 15 mg Bumetanide Dibenzyamide to male and female Dogs on a log-linear scale.

[0049] FIG. 6A shows the individual animal Bumetanide Dibenzyamide concentration-time profiles following IR administration of 30 mg Bumetanide Dibenzyamide to male and female Dogs on a linear scale.

[0050] FIG. 6B shows the individual animal Bumetanide Dibenzyamide concentration-time profiles following IR administration of 30 mg Bumetanide Dibenzyamide to male and female Dogs on a log-linear scale.

[0051] FIG. 7A shows the mean Bumetanide Dibenzyamide concentration-time profiles following various routes of administration of 30 mg Bumetanide Dibenzyamide to male and female dogs on a linear scale.

[0052] FIG. 7B shows the mean Bumetanide Dibenzyamide concentration-time profiles following various routes of administration of 30 mg Bumetanide Dibenzyamide to male and female dogs on a log-linear scale.

[0053] FIG. 8A shows the mean Bumetanide Dibenzyamide and Bumetanide plasma concentration-time profiles following IV bolus administration of 10 mg/kg Bumetanide Dibenzyamide to male rats on a linear scale.

[0054] FIG. 8B shows the mean Bumetanide Dibenzyamide and Bumetanide plasma concentration-time profiles following IV bolus administration of 10 mg/kg Bumetanide Dibenzyamide to male rats on a semi-log scale.

[0055] FIG. 9A shows the mean Bumetanide Dibenzylamide and Bumetanide plasma concentration-time profiles following oral administration of 30 mg/kg Bumetanide Dibenzylamide to male rats on a liner scale.

[0056] FIG. 9B shows the mean Bumetanide Dibenzylamide and Bumetanide plasma concentration-time profiles following oral administration of 30 mg/kg Bumetanide Dibenzylamide to male rats on a semi-log scale.

[0057] FIG. 10A shows the mean Bumetanide Dibenzylamide and Bumetanide plasma concentration-time profiles following IV (10 mg/kg) and oral (30 mg/kg) doses Bumetanide Dibenzylamide to male rats on a liner scale.

[0058] FIG. 10B shows the mean Bumetanide Dibenzylamide and Bumetanide plasma concentration-time profiles following IV (10 mg/kg) and oral (30 mg/kg) doses Bumetanide Dibenzylamide to male rats on a semi-log scale.

[0059] FIG. 11A shows the Bumetanide plasma concentration-time profiles following oral administration of 30 mg/kg Bumetanide Dibenzylamide to dogs on a liner scale.

[0060] FIG. 11B shows the Bumetanide plasma concentration-time profiles following oral administration of 30 mg/kg Bumetanide Dibenzylamide to dogs a semi-log scale.

[0061] FIG. 12 shows the X-ray diffractogram analysis of bumetanide dibenzylamide.

[0062] FIG. 13 shows the DSC analysis of bumetanide dibenzylamide.

[0063] FIG. 14 shows the TGA analysis of bumetanide dibenzylamide.

[0064] FIG. 15 shows the DVS analysis of bumetanide dibenzylamide.

[0065] FIG. 16 shows the PSD analysis of bumetanide dibenzylamide.

[0066] FIG.17 shows the morphology of Bumetanide Dibenzylamide under 40X magnification.

[0067] FIG. 18 is a listing of indications, in order to reference variability in classification over time.

[0068] FIG. 19 is a tabulated calculation of the Permeability of Test Compounds in Caco-2 Assay of Example 9.

[0069] FIG. 20 is a tabulated depiction of LCMS data from the Permeability of Test Compounds in Caco-2 Assay of Example 9.

[0070] FIG. 21 is a tabulated depiction of the Lucifer Yellow Rejection Assay for Monolayer Integrity Test associated with the Permeability of Test Compounds in Caco-2 Assay of Example 9.

[0071] FIG. 22A, 22B, and 22C likewise are (A) a tabulated calculation of the Permeability of Test Compounds in Caco-2 Assay of Example 9; (B) a tabulated depiction of LCMS data from the Permeability of Test Compounds in Caco-2 Assay of Example 9; and (C) a tabulated depiction of the Lucifer Yellow Rejection Assay for Monolayer Integrity Test associated with the Permeability of Test Compounds in Caco-2 Assay of Example 9.

[0072] FIG. 23 shows the blood concentration of the formulated (black triangles) and unformulated (grey circles) Bumetanide Morpholinoamide at 2 hrs, 6 hrs, and 12 hrs post treatment.

[0073] FIG. 24 illustrates formulated and unformulated Bumetanide Morpholinoamide blood concentration time points prior to 2hrs using an exponential fit to the data.

[0074] FIG. 25 shows the blood concentration of the formulated (black triangles) and unformulated (grey circles) Bumetanide Diethylamide at 2 hrs, 6 hrs, and 12 hrs post treatment.

DETAILED DESCRIPTION

[0075] The terms “active ingredient”, “active pharmaceutical ingredient,” and “API” as used herein refer to a pharmaceutical agent, active ingredient, compound, or substance, compositions, or mixtures thereof, that provide a pharmacological, often beneficial, effect.

[0076] The term “dose” as used herein denotes any form of the active ingredient formulation that contains an amount sufficient to produce a therapeutic effect with a single administration.

[0077] The term “dosage” as used herein refers to the administering of a specific amount, number, and frequency of doses over a specified period-of-time, typically one (1) day.

[0078] The terms “active pharmaceutical ingredient load” or “drug load” as used herein refers to the quantity (mass) of the active pharmaceutical ingredient comprised in a single soft capsule fill.

[0079] The terms “formulation” or “pharmaceutical composition” or “composition” as used herein refers to the drug in combination with pharmaceutically acceptable excipients.

[0080] The term mean “particle size distribution” (PSD) as used herein refers to the mean particle size from a statistical distribution of a range of particle sizes as described herein. The distribution may be a Gaussian, normal distribution, or a non-normal distribution.

[0081] The terms such as “d90,” “d50,” and “d10” refer to the percentage (e.g., 90%, 50%, or 10%, respectively) of particle sizes that are less than a specified size, range, or distribution. For example, “ $d_{90} \leq 100 \mu\text{m}$ ” as means that 90% of the particle sizes within a distribution of particles are less than or equal to 100 μm .

[0082] As used herein, the term “patient” refers to any subject including mammals and humans. The patient may have a disease or suspected of having a disease and as such is being treated with a drug. In some instances, the patient is a mammal, such as a human, non-human primate, dog, cat, horse, cow, goat, pig, rabbit, rat, mouse, or a premature neonate, neonate, infant, juvenile, adolescent, or adult thereof. In some instances, the term “patient,” as used herein, refers to a human (e.g., a man, a woman, or a child). In some instances, the term “patient,” as used herein, refers to laboratory animal of an animal model study. The patient or subject may be of any age, sex, or combination thereof.

[0083] The terms “biological sample” or “sample” as used herein refers to a sample obtained or derived from a patient. By way of example, a biological sample comprises a

material selected from the group consisting of body fluids, blood, whole blood, plasma, serum, mucus secretions, saliva, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF), urine, fluids of the eye (e.g., vitreous fluid, aqueous humor), lymph fluid, lymph node tissue, spleen tissue, bone marrow, and fluid from the auditory cavity.

[0084] The term “treating” refers to administering a therapy in an amount, manner, or mode effective (e.g., a therapeutic effect) to improve a condition, symptom, disorder, or parameter associated with a disorder, or a likelihood thereof.

[0085] The term “prophylaxis” refers to preventing or reducing the progression of a disorder, either to a statistically significant degree or to a degree detectable to one skilled in the art.

[0086] The terms “essentially” or “substantially” as used herein mean to a great or significant extent, but not completely.

[0087] The term “about” as used herein refers to any values, including both integers and fractional components that are within a variation of up to $\pm 10\%$ of the value modified by the term “about.”

[0088] Also described herein are pharmaceutical compositions and dosage forms comprising one or more agents that reduce the rate by which the compositions described herein as active ingredients will decompose. Such agents, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, salts, sugars, etc.

[0089] The phrase ‘solubilizer’ is used to refer to an ingredient or group of ingredients that helps solubilize the composition or part of the composition.

[0090] The phrases and terms “can be administered by injection”, “injectable”, or “injectability” refer to a combination of factors such as a certain force applied to a plunger of a syringe containing the formulations described herein and at a certain temperature, a needle of a given inner diameter connected to the outlet of such syringe, and the time

required to extrude a certain volume of the Bumetanide Dibenzylamide composition from the syringe through the needle.

[0091] The range for each ingredient in the described formulation represent the space in which a suitable alternative(s) may be obtained in combination with the other ingredients in ratios adjusted to total 100% w/w. The ranges provided are estimates based on the available data.

[0092] One embodiment described herein, is a pharmaceutical composition comprising Bumetanide Dibenzylamide. In one aspect, the composition comprises any of the formulations shown in the Tables or Examples described herein. Any of the components in the formulations described herein, shown in the Tables, or illustrated in the Examples can be increased, decreased, combined, substituted, or omitted to provide for a formulation comprising about 100% by weight. Such compositions are hereby disclosed as if they were expressly disclosed herein.

[0093] One embodiment described herein is a pharmaceutical composition comprising Bumetanide Dibenzylamide, and one or more solubilizers. Another embodiment described herein is a pharmaceutical composition comprising Bumetanide Dibenzylamide. Another embodiment described herein is a pharmaceutical composition further comprising one or more additional solvents. Another embodiment described herein is a pharmaceutical composition further comprising one or more surfactants, co-surfactants, emulsifying agent or wetting agent. Another embodiment described herein is a pharmaceutical composition consisting essentially of Bumetanide Dibenzylamide. Another embodiment described herein is a pharmaceutical composition consisting essentially of aqueous Bumetanide Dibenzylamide. Another embodiment described herein is a pharmaceutical composition comprising Bumetanide Dibenzylamide, and one or more solubilizers. Another embodiment described herein is a pharmaceutical composition consisting essentially of Bumetanide Dibenzylamide, and one or more solubilizers. In one aspect, the composition is a dry powder compressed into a tablet. In one aspect, the composition is a dry powder filled into a capsule. In one aspect, the composition is a dry powder extruded into a film. In one aspect, the composition is a dry powder extruded into a tablet. One embodiment described

herein is a pharmaceutical composition comprising about 2.5 mg to about 42 mg of bumetanide dibenzylamide.

[0094] One embodiment described herein is a pharmaceutical composition formulated as an oral capsule. In one aspect, the composition comprises up to about 0.25%w/w to about 15%w/w of Bumetanide Dibenzylamide, and one or more solubilizers. In one aspect, the solubilizer is a co-solvent. In one aspect, the solubilizer is a surfactant. In one aspect, the solubilizer comprises triglycerides. In one aspect, the triglycerides comprises medium chain triglycerides. In one aspect, the triglycerides comprises long chain triglycerides. In one aspect, the triglycerides comprises a mixture of medium and long chain triglycerides. In one aspect, the triglycerides comprises polyoxylglycerides. In one aspect, the polyoxylglycerides is selected from a group consisting Lauroyl Polyoxylglycerides, Linoleoyl Polyoxylglycerides, Oleoyl Polyoxylglycerides, Stearoyl Polyoxylglycerides, Caprylocaproyl Polyoxylglyceride, and any combination thereof. In one aspect, the triglyceride comprises a non-ionic surfactant, solubilizer, emulsifying agent. In one aspect, the long chain triglycerides are selected from a group consisting of Polyoxyl 35 Castor Oil (Kolliphor EL), Glyceryl Monolinoleate (Maisine CC), and any combination thereof. In one aspect, the medium chain triglycerides are selected from a group consisting of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Phosphatidylcholine (Phosal 75 SA), Caprylic/Capric Triglyceride (Captex 300), Lauroyl Polyoxyl-32 Glycerides (Gelucire 44/14), Sorbitan Ester (Span 80), and any combination thereof.

[0095] In one aspect, described herein is a pharmaceutical composition comprising about 0.1 %w/w to about 99.75 %w/w of one or more solubilizers. In one aspect, the solubilizer comprises soybean oil. In one aspect, the solubilizer is in the oil phase. In one aspect, the solubilizer is selected from a group consisting of Arachis oil, soybean oil, castor oil, corn oil, safflower oil, olive oil, apricot kernel oil, sesame oil, cotton-seed oil, sunflower seed oil, palm oil and rapeseed oil, Maisine 35-1, Maisine CC (Glyceryl Monolinoleate), and any combination thereof. In one aspect, the solubilizer comprises a co-solvent. In one aspect, the solubilizer is selected from a group consisting of Propylene Glycol, Capryol™ 90 (Propylene glycol monocaprylate), Lauroglycol™ 90 (Propylene glycol monolaurate), Glycerin, Polyethylene Glycol, and any combination thereof. In one aspect, the solubilizer comprises an

antioxidant. In one aspect, the solubilizer is selected from a group consisting of alpha tocopherol, ascorbyl palmitate, ascorbic acid, butylated hydroxyanisole, butylated hydroxytoluene, and any combination thereof. In one aspect, the solubilizer comprises an antimicrobial preservative, a solvent, and a water-soluble co-solvent. In one aspect, the solubilizer comprises a solvent, and a water-soluble co-solvent. In one aspect, the solubilizer is selected from a group consisting Ethanol, Propylene Glycol, Propylene Glycol 300, Propylene Glycol 400, Propylene Glycol 600, Oleyl Alcohol, and any combination thereof. In one aspect, the solubilizer is water. In one aspect, the solubilizer is any diluent.

[0096] In one aspect, described herein is a pharmaceutical composition comprising about 0.5 %w/w to about 1.8 %w/w of Bumetanide Dibenzyamide. In one aspect, described herein is a pharmaceutical composition comprising about 9 mg of Bumetanide Dibenzyamide per capsule to about 12 mg of Bumetanide Dibenzyamide per capsule. In one aspect, described herein is a pharmaceutical composition comprising about 0 %w/w to about 1.8 %w/w of Bumetanide Dibenzyamide, about 10 %w/w to about 45 %w/w of Polyoxyl 35 Castor Oil (Kolliphor EL), about 15 %w/w to about 65 %w/w of Glyceryl Monolinoleate (Maisine CC), about 15 %w/w to about 65 %w/w of Soybean Oil, about 0 %w/w to about 15 %w/w of Ethanol, and about 0%w/w to about 0.13 %w/w of Butylated Hydroxytoluene. In one aspect, described herein is a pharmaceutical composition comprising about 1.75 %w/w of Bumetanide Dibenzyamide, about 32.37 %w/w of Polyoxyl 35 Castor Oil (Kolliphor EL), about 31.30 %w/w of Glyceryl Monolinoleate (Maisine CC), about 31.30 %w/w of Soybean Oil, about 3.25 %w/w of Ethanol, and about 0.3 %w/w of Butylated Hydroxytoluene.

[0097] One embodiment described herein is a pharmaceutical composition formulated as a nasal solution. In one aspect, the composition comprises Bumetanide Dibenzyamide in a solvent system. In one aspect, the composition comprises about 3 ml of solvent and from about 28 mg of Bumetanide Dibenzyamide to about 32 mg of Bumetanide Dibenzyamide. In one aspect, the solubilizer comprises triglycerides. In one aspect, the triglycerides comprises medium chain triglycerides. In one aspect, the triglycerides comprises long chain triglycerides. In one aspect, the triglycerides comprises a mixture of medium and long chain triglycerides. In one aspect, the triglycerides comprises polyoxylglycerides. In one aspect,

the polyoxylglycerides is selected from a group consisting Lauroyl Polyoxylglycerides, Linoleoyl Polyoxylglycerides, Oleoyl Polyoxylglycerides, Stearoyl Polyoxylglycerides, Caprylocaproyl Polyoxylglyceride, and any combination thereof.

[0098] In one aspect, the triglyceride comprises a non-ionic surfactant, solubilizer, emulsifying agent. In one aspect, the solubilizer comprises Caprylocaproyl Polyoxylglycerides (Labrasol ALF). In one aspect, the solvent system comprises one or more solubilizers. In one aspect, the solubilizer comprises Caprylocaproyl Polyoxylglycerides (Labrasol ALF), and water. In one aspect, the solubilizer comprises Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Propylene Glycol, and water. In one aspect, the solubilizer comprises Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Propylene Glycol, PEG-400, and water. In one aspect, the solubilizer comprises Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Propylene Glycol, PEG-400, Vitamin E TPGS, and water. In one aspect, the solubilizer comprises Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Propylene Glycol, PEG-400, Vitamin E TPGS, Ethanol, and water. In one aspect, the solubilizer comprises about 50g of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), per 50g of solvent. In one aspect, the solubilizer comprises about 25g of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), and about 25g of water per 50 g of solvent. In one aspect, the solubilizer comprises about 6g of Propylene Glycol, about 40g of PEG-400, and about 4g of water per 50 g of solvent. In one aspect, the solubilizer comprises about 5g of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 6g of Propylene Glycol, about 35g of PEG-400, and about 4g of water per 50 g of solvent. In one aspect, the solubilizer comprises about 10g of Propylene Glycol, about 35g of PEG-400, about 0.5g of Vitamin E TPGS, and about 4.5g of water per 50g of solvent. In one aspect, the solubilizer comprises about 5g of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 10g of Propylene Glycol, about 28.5g of PEG-400, about 0.5g of Vitamin E TPGS, about 1g of Ethanol, and about 4.5g of water per 50 g of solubilizer. In one aspect, the solubilizer comprises glycofurol. In one aspect, the solubilizer comprises a penetration agent, and a solvent. In one aspect, the solubilizer comprises ethyl oleate. In one aspect, the solubilizer comprises an oleaginous vehicle, solvent, and a solvent.

[0099] In one aspect, the pharmaceutical composition comprises about 3 %w/v of Bumetanide Dibenzyamide, about 11 %w/w of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 13.26 %w/v of Propylene Glycol, about 73.94%w/v of PEG-400, and about 8.8 %w/w of water. In one aspect, the pharmaceutical composition comprises from about 0.01 %w/w to about 40 % w/w of Bumetanide Dibenzyamide, from about 5 %w/w to about 100 % w/w of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), from about 4 %w/w to about 20 % w/w of Propylene Glycol, from about 50 %w/w to about 80 % w/w of PEG-400, and from about 0 %w/w to about 10 % w/w of water. In one aspect, the pharmaceutical composition comprises about 2.73 %w/w of Bumetanide Dibenzyamide, about 8%w/w of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 15 %w/w of Propylene Glycol, about 69.27 %w/w of PEG-400, and about 5 %w/w of water. In one aspect, the pharmaceutical composition comprises about 2.73 %w/w of Bumetanide Dibenzyamide, about 16%w/w of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 20 %w/w of Propylene Glycol, about 54.27 %w/w of PEG-400, and about 7 %w/w of water. In one aspect, the pharmaceutical composition comprises about 2.73 %w/w of Bumetanide Dibenzyamide, about 5%w/w of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 4%w/w of Propylene Glycol, about 78.27 %w/w of PEG-400, and about 10 %w/w of water.

[00100] One embodiment described herein is a pharmaceutical composition formulated as a rectal paste. One embodiment described herein is a composition formulated as a rectal gel. In one aspect, the composition is formulated with a target of about 6mg Bumetanide Dibenzyamide per gram of the composition based on a target dose of about 30mg of Bumetanide Dibenzyamide in an amount of about 5g of the composition. In one aspect, the composition is formulated with a different target dose of Bumetanide Dibenzyamide. In one aspect, the paste is determined to be a 100% non-aqueous formulation in case the drug substance exhibited some instability in water. In one aspect, the rectal gel is formulated to comprise about 0.6 %w/w Bumetanide Dibenzyamide, about 10 %w/w Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 9.15 %w/w Propylene Glycol, about 53.84 %w/w Polyethylene Glycol 600, about 3.6 %w/w Polyvinylpyrrolidone (K30), about 2.4 %w/w Poloxamer 407 (P407), about 0.41 %w/w Sodium Carboxymethyl Cellulose (CMC), and

about 20 %w/w water. In one aspect, the rectal paste is formulated to comprise about 0.6 %w/w Bumetanide Dibenzyamide, about 10 %w/w Caprylocaproyl Polyoxylglycerides (Labrasol ALF), about 9.15 %w/w Propylene Glycol, about 66.75 %w/w Polyethylene Glycol 600, about 3.6 %w/w Polyvinylpyrrolidone (K30), about 2.4 %w/w Poloxamer 407 (P407), and about 7.5 % w/w Polyethylene Glycol 3350.

[00101] One embodiment described herein is a pharmaceutical composition formulated as a sublingual tablet. In one aspect, the composition formulated is targeted to have about 30mg Bumetanide Dibenzyamide per tablet. In one aspect, a small tablet size is used. In one aspect, wetting and or dissolving of the composition occurs within 30 seconds. In one embodiment, the sublingual tablet is formulated to comprise Bumetanide Dibenzyamide, one or more wetting agent, and one or more super disintegrate. In one aspect, the sublingual tablet is formulated to comprise about 15 %w Bumetanide Dibenzyamide, about 20 %w Ceolus KG (microcrystalline cellulose), about 51 %w Mannogem EZ (spray dried mannitol), about 7 %w Polyplasdone XL (super disintegrate), about 3%w Poloxamer 407 (wetting agent), about 1.5 %w Citric Acid Monohydrate, about 1 %w Cabosil M5P (fumed silica), and about 1.5 %w Magnesium Stearate. In one embodiment, the sublingual tablet is formulated to comprise Bumetanide Dibenzyamide, one or more water dispersible surfactant, one or more wetting agent, and one or more super disintegrate. In one aspect, the sublingual tablet is formulated to comprise about 7.4 %w Bumetanide Dibenzyamide, about 9.9 %w Lauroyl Polyoxyl-32 Glycerides (Gelucire 44/14, a water dispersible surfactant), about 9.9 %w Sorbitan Ester (Span 80, a water dispersible surfactant), about 14.8 %w Neusilin US2 (Magnesium Aluminometasilicate), about 0.5%w/w Poloxamer 407 (wetting agent), about 0.7 %w Citric Acid Monohydrate, about 2 %w Cabosil M5P (Fumed Silica), about 54.3 %w Polyplasdone XL (Super Disintegrate), and about 0.5 %w Magnesium Stearate.

[00102] One embodiment described herein, the preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) are for use in treating seizures (e.g., partial onset seizures), epilepsy, and/or other indications such as neuropathic pain by modulating or disrupting the synchrony of neuronal population activity in areas of heightened synchronization by reducing the activity of NKCC co-

transporters without having a diuretic effect. One embodiment described herein, are preferred treatment agents and methods of the present disclosure for treating seizures that cannot be controlled by existing pharmacotherapeutics such as uncontrolled seizures, intractable seizures, refractory seizures, drug resistant seizures, or medically resistant seizures. One embodiment described herein, are preferred treatment agents and methods of the present disclosure for treating epilepsy syndromes such as Angelman syndrome, benign rolandic epilepsy, CDKL5 disorder, childhood absence epilepsy, Dravet syndrome, GLUT1 deficiency syndrome, hypothalamic hamartoma, infantile spasms (also known as West syndrome), Lennox-Gastaut, PCDH19, progressive myoclonic epilepsy, Rasmussen's encephalitis, ring chromosome 20 syndrome, or reflex epilepsies.

[00103] One embodiment described herein, the preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) are for use in treating the epilepsy and/or neurological syndromes that are specific to children including but not limited to Dravett syndrome, infantile spasms, Landau-Kleffner Syndrome, Lennox-Gastaut Syndrome, Rasmussen Syndrome, Benign Rolandic Epilepsy, Benign Occipital Epilepsy, Childhood Absence Epilepsy, Juvenile Myoclonic, Rett Syndrome, Angelman Syndrome, Tuberous Sclerosis, and/or Sturge Weber Syndrome. One embodiment described herein, the preferred treatment agents and methods of the present disclosure are for use in treating epilepsy and/or neurological syndromes that may be observed in adults or children.

[00104] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) for use in treating one or more indications listed in Figure 18 (Fisher et al.) and for treating whatever terms might replace the old and current terms describing the indications listed in Figure 18 in the future.

[00105] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) for use in treating comorbidities of epilepsy or seizures such as psychiatric disorders such as depression, anxiety disorders, attention deficit hyperactivity disorder (ADHD), schizophrenia-like interictal psychosis, autism, as well as suicidal behavior, sleep disorders, autism spectrum

disorders, migraines, postictal headaches, depression, anxiety, psychosis, Attention Deficit Disorder (ADD) and Attention Deficit/Hyperactivity Disorder (ADHD), or mental retardation.

[00106] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) for use in treating migraines or tinnitus. In one aspect, the preferred treatment agents and methods of the present disclosure may be used to treat migraines with or without aura in adults. In another aspect, the preferred treatment agents and methods of the present disclosure may be used for acute treatment of migraine with auro, acute treatment of migraine without auro, or for chronic treatment for the prevention of migraine with our without aura.

[00107] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) for use in treating mild, moderate, or severe anxiety. In one aspect, the preferred treatment agents and methods of the present disclosure may be used for the acute and maintenance treatment of Major Depressive disorder (MDD) in adults and adolescents aged 12-17 years, or for the acute treatment of Generalized Anxiety Disorder (GAD) in adults. In one aspect, the preferred treatment agents and methods of the present disclosure may be used for the acute and maintenance treatment of Obsessive Compulsive Disorder (OCD), acute and maintenance treatment of Bulimia Nervosa, or acute treatment of Panic Disorder (PD) with or without agoraphobia. In another aspect, the preferred treatment agents and methods of the present disclosure may be used for treating acute depressive episodes associated with bipolar I disorder or for treating treatment resistant depression.

[00108] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) may be for use in treating obsessions and compulsions in patients with Obsessive Compulsive Disorder (OCD), Major Depressive Disorder (MDD), Panic Disorder (PD), Social Anxiety Disorder (SAD), Pre-menstrual dysphoric disorder (PMDD), or Posttraumatic Stress Disorder (PTSD). In one aspect, the obsessions or compulsions may cause marked distress, be time-consuming, or significantly interfere with social or occupational functioning, in order to meet the

DSM-III-R (circa 1989) diagnosis of OCD. Obsessions may be recurrent, persistent ideas, thoughts, images, or impulses that are egodystonic. Compulsions may be repetitive, purposeful, and/or intentional behaviors performed in response to an obsession or performed in a stereotyped fashion. Compulsions may be recognized by the person as excessive or unreasonable

[00109] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) for use in treating psychoneurotic patients with mild, moderate, or severe depression, anxiety associated with depression, anxiety as associated with alcoholism, depression and/or anxiety as associated with organic disease, psychotic depressive disorders with associated anxiety including involuntional depression and manic-depressive disorders. In one aspect, the preferred treatment agents and methods of the present disclosure may be used to target symptoms of psychoneurosis such as anxiety, tension, depression, somatic symptoms and concerns, sleep disturbances, guilt, lack of energy, fear, apprehension, and worry.

[00110] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) for use in treating depressive illness in patients with depressive neurosis (dysthymic disorder), manic-depressive illness, or with major depressive disorder. In one aspect, the preferred treatment agents and methods of the present disclosure may be used for short-term, long-term and maintenance treatment of Major Depressive Disorder (MDD), Generalized Anxiety Disorder, Diabetic Peripheral Neuropathic Pain (DPNP), Fibromyalgia (FM), or Chronic Musculoskeletal Pain.

[00111] One embodiment described herein, are treatment agents and methods of the present disclosure for treating a progressive neurodegenerative disorder, including, for example, Alzheimer's disease. In one aspect, the preferred treatment agents and methods of the present disclosure may be used for treatment or cessation of disease progression for one or more of Alzheimer's disease, amyotrophic lateral sclerosis, Friedrich ataxia, Huntington's disease, Lewy Body disease, Parkinson's disease, or spinal muscular atrophy.

[00112] One embodiment described herein, are preferred treatment agents and methods of the present disclosure comprising use as a monotherapy or an adjunctive therapy. In one aspect, the

preferred treatment agents and methods of the present disclosure may be used as a monotherapy for adults. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as an adjunctive therapy with additional therapy agents for adults. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as a monotherapy for pediatric patients 2 years of age and older. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as an adjunctive therapy with additional therapy agents for pediatric patients 2 years of age and older. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as a monotherapy for pediatric patients less than 2 years of age. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as an adjunctive therapy with additional therapy agents for pediatric patients less than 2 years of age.

[00113] One embodiment described herein, are preferred treatment agents and methods of the present disclosure (including all aspects and embodiments of those agents and methods) comprising use as a monotherapy or an adjunctive therapy. In one aspect, the preferred treatment agents and methods of the present disclosure may be used as a monotherapy for adults. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as an adjunctive therapy with additional therapy agents for adults. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as a monotherapy for pediatric patients 2 years of age and older. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as an adjunctive therapy with additional therapy agents for pediatric patients 2 years of age and older. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as a monotherapy for pediatric patients less than 2 years of age. In another aspect, the preferred treatment agents and methods of the present disclosure may be used as an adjunctive therapy with additional therapy agents for pediatric patients less than 2 years of age.

[00114] The effective amount of an active pharmaceutical ingredient to be administered therapeutically will depend, for example, upon the therapeutic context and objectives. One having ordinary skill in the art will appreciate that the appropriate dosage levels for treatment will vary depending, in part, upon the concentration of the Bumetanide

Dibenzylamide composition (or a composition comprising any other prodrug of Bumetanide described herein), the dosing regimen for which the Bumetanide Dibenzylamide composition (or a composition comprising any other prodrug of Bumetanide described herein) is being used, the route of administration, and the subject's size (body weight or body surface area) and condition (the age and general health) of the patient. Accordingly, the dosage may be titrated to obtain the optimal therapeutic effect.

[00115] The frequency of dosing will depend upon the pharmacokinetic parameters of the therapeutic agent incorporated into the Bumetanide Dibenzylamide composition (or a composition comprising any other prodrug of Bumetanide described herein) being used. The composition can be administered as a single dose, as two or more doses (which may or may not contain the same amount of the Bumetanide Dibenzylamide, or any other prodrug of Bumetanide described herein) over time, or as a continuous infusion of an injection formulation via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. A sublingual tablet may also be used for oral administration). Appropriate dosages can be ascertained through use of appropriate dose-response data.

[00116] Refinement opportunities may include extended or controlled release oral capsule or tablet or use of a transdermal formulation. Intramuscular data shown below supports the development of a potential transdermal therapy. The intramuscular data shows that Bumetanide Dibenzylamide or any other prodrug of Bumetanide described herein can be absorbed through the microvessels of the muscle into the circulation, and thus avoiding first-pass metabolism. Hence the Bumetanide Dibenzylamide should also be absorbed by the dermal microvessels, lending itself to a transdermal formulation.

[00117] The Bumetanide Dibenzylamide composition (or a composition comprising any other prodrug of Bumetanide described herein) can be administered, for example, 1x, 2x, 3x, 4x, 5x, 6x, or even more times per day. One or more doses can be administered, for example, for 1, 2, 3, 4, 5, 6, 7 days, or even longer. One or more doses can be administered, for example, for 1, 2, 3, 4 weeks, or even longer. One or more doses can be administered, for example, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1 year, 2 years, 3 years, 4 years,

5 years, over 5 years, a decade, multiple decades, or even longer. One or more doses can be administered at a regular interval until the subject or subject in need thereof, does not require treatment or prophylaxis of epilepsy. In one aspect, the doses maybe administered orally. In one aspect, the doses maybe administered sublingually. In one aspect, the doses maybe administered intravenously. In one aspect, the doses maybe administered intrarectally. In one aspect, the doses maybe administered intramuscularly. In one aspect, the doses maybe administered intranasally. In one aspect, the doses maybe administered subcutaneously.

[00118] In one embodiment, the pharmaceutical composition described herein is administered in one or multiple doses simultaneously. For example, two or more identical doses are administered at one time. In another embodiment, two or more different doses are administered at one time. Such dual or different simultaneous doses can be used to provide an effective amount of the pharmaceutical composition to a subject in need thereof.

[00119] In one embodiment, the pharmaceutical compositions described herein may be used to treat, prevent, retard the progression of, delay the onset, ameliorate, reduce the symptoms of, or prophylaxis of epilepsy.

[00120] In one embodiment, the Bumetanide Dibenzyamide composition (or a composition comprising any other prodrug of Bumetanide described herein) described herein is sufficiently dosed in the composition to provide a therapeutically effective amount in one application. In one aspect, one application of Bumetanide Dibenzyamide composition (or a composition comprising any other prodrug of Bumetanide described herein) is sufficient for about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, one month, 2 months, 3 months, 4 months, 6 months, 9 months, one year, 2 years, 3 years, 4 years, or even longer. In one aspect, one application of Bumetanide Dibenzyamide composition (or a composition comprising any other prodrug of Bumetanide described herein) is given more than once per day.

[00121] Another embodiment, the Bumetanide Dibenzyamide composition, (or a composition comprising any other prodrug of Bumetanide described herein) described

herein is provided as a single dose, meaning that the container in which it is supplied contains one pharmaceutical dose. In another embodiment, the composition is provided as a multiple dose composition, meaning that it contains more than one therapeutic dose. Preferably, a multiple dose composition contains at least 2 doses. Such multiple dose Bumetanide Dibenzylamide composition (or a composition comprising any other prodrug of Bumetanide described herein) either can be used for different subjects in need thereof or is intended for use in one subject, wherein the remaining doses are stored after the application of the first dose until needed. In another embodiment, the Bumetanide Dibenzylamide composition (or a composition comprising any other prodrug of Bumetanide described herein) is comprised in one or more containers.

[00122] Crucial to the efficacy of any treatment by a pharmaceutical composition is the overall systemic bioavailability of the pharmaceutical composition used for said treatment. Surprisingly, lipid formulations may be used to increase the bioavailability and/or lymphatic absorption of a pharmaceutical composition. LogP is one measure of lipophilicity, and is the octal: water partition coefficient expressed as a log ratio of molecule in octanol relative to water after mixing. The LogP of Bumetanide is about 2.61, the logP of Bumetanide Diethylamide is 3.11, and the logP of Bumetanide Dibenzylamide is about 5.9. A LogP of 3 indicates a 1000-fold greater concentration in octanol than water, so Bumetanide Dibenzyl amide is about 1000 – to about 10,000-fold more lipophilic than Bumetanide.

[00123] As described herein, it is found that Bumetanide Dibenzylamide or any other prodrug of Bumetanide described herein disrupts the synchrony of neuronal population activity in areas of heightened synchronization. The dosage compositions of Bumetanide Dibenzylamide were developed targeting four (4) routes of administrations in attempt to bypass first-pass metabolism for of the composition and increase overall systemic bioavailability. The routes of administration were chosen based upon their potential to maximize the bioavailability of Bumetanide Dibenzylamide and to produce measurable systemic concentrations of Bumetanide Dibenzylamide. Since Bumetanide Dibenzylamide has been shown to be susceptible to high first-pass metabolism by the liver, the routes of administration were selected to avoid hepatic metabolism.

[00124] As described below, the pharmacokinetic profiles and bioavailability of the composition were evaluated following oral (PO), sublingual (SL), intranasal (IN), intrarectal (IR), subcutaneous (SC), intravenous (IV), and intramuscular (IM) dose administration. It was surprisingly found that oral administration of Bumetanide Dibenzyamide formulated with triglycerides bypasses first-pass metabolism of the composition by the liver.

[00125] In one embodiment described herein, the pharmacokinetic profiles and bioavailability of Bumetanide Dibenzyamide following oral (PO), sublingual (SL), intranasal (IN), intrarectal (IR), subcutaneous (SC), and intramuscular (IM) dose administration is evaluated in a crossover design in male and female Beagle dogs. The IN, IM, and SC dose level is about 15 mg of Bumetanide Dibenzyamide and drug exposure is the highest following IM administration followed by similar exposures for SC and IN administration. The PO, SL, and IR dose level is about 30 mg of Bumetanide Dibenzyamide and the drug exposure is much higher for PO administration followed by IR administration. The Bumetanide Dibenzyamide concentration following SL administration is relatively low.

[00126] Study Design

[00127] Male and female Beagle dogs are assigned to two groups with 2 males and 2 females in each group. The study is conducted in 3 legs with Leg 1 consisting of IM and SL administration, Leg 2 consisting of IN and PO administration, and Leg 3 consisting of SC and IR administration. As illustrated in Table 1, group 1 animals receive IM, IN and SC dosing, and Group 2 animals receive SL, PO, and IR administration, each in a crossover design. Group 1 animals receive about 15 mg doses and Group 2 receive about 30 mg doses of Bumetanide Dibenzyamide.

[00128] Table 1: Group Assignments and Treatment Information

Leg #	Group #	Dose Route	N		Dose Level (mg)	Dose Concentration (mg/mL)	Dose Volume (mL)
			M	F			
1	1	IM	2	2	15	30	0.5
	2	SL	2	2	30	NA	1 tablet
2	1	IN	2	2	15	30	0.5*
	2	PO	2	2	30	NA	3 capsules
3	1	SC	2	2	15	30	0.5
	2	IR	2	2	30	60	0.5

[00129] Dosing Procedures

[00130] Administration of Bumetanide Dibenzyamide composition is as described herein. During intranasal administration, the composition is administered by gently tilting the animals head back and the intranasal dose is delivered via a syringe and atomizer. During sublingual administration, the composition is administered via a quick dissolving tablet. The tablet is placed in the sublingual space and the mouth held shut for approximately 60-90 seconds. After one minute, the mouth of the animal is opened to confirm that the tablet has fully dissolved. During intramuscular administration, the composition is administered into the bicep femoris (rear thigh). The dose site is gently shaved prior to dosing. A syringe with a 25g needle is utilized. During oral administration, the composition is administered via three capsules placed at the back of the animal's mouth and the placement is followed by a 10mL drinking water flush to assure that the animal swallowed the capsules. During subcutaneous administration, the composition is administered into the dorsal subcutaneous space directly at the scruff of the neck of the animal. The dose site is gently shaved prior to

dosing. A syringe with a 25g needle is utilized. During intrarectal administration, the composition is administered by gently placing a 1cc syringe in the rectum of the animal.

[00131] All animals are fasted for approximately 12 hours prior to dosing. Animals are fed four hours post dosing with water offered ad libitum throughout the study. All animals completed the study. Body weights remained stable and no adverse reactions are noted throughout the study with all animals showing normal behavior following dose administrations. Blood and urine samples are collected out to 24 hours. Table 2 contains information regarding the sex and body weights of the animals in the study.

[00132] Table 2: Sex and Body Weights (kg) of Study Animals

Animal #	Sex	Leg 1	Leg 2	Leg 3
2960045 (#1)	Male	13.2	13.5	13.5
2923344 (#2)	Male	13.5	13.5	13.3
CJDCDD (#3)	Female	12.0	12.0	11.3
CJCCJW (#4)	Female	11.4	11.2	11.0
2972702 (#5)	Male	12.2	13.0	12.5
2974674 (#6)	Male	13.1	13.1	13.1
CJACUH (#7)	Female	8.7	8.3	8.3
CJCCBU (#8)	Female	10.0	9.4	9.5

[00133] Plasma Sample Analysis for Bumetanide Dibenzylamide and Bumetanide

[00134] Blood sample are collected from the jugular vein, or other suitable vessel via direct venipuncture, placed into a chilled tube containing K2EDTA as the anticoagulant, and inverted several times to mix. Blood samples are kept on wet ice until centrifugation. Plasma is harvested and stored on dry ice until placed in a freezer, set to maintain 80°C, until analysis.

[00135] Plasma samples are analyzed for Bumetanide Dibenzyamide and Bumetanide concentration using a LC-MS/MS method. The assay is verified with respect to standard bioanalytical methodology including acceptable accuracy and precision based upon quality control sample analysis and results. The range of the method for both analytes is from about 0.250 ng/mL to about 250 ng/mL.

[00136] Plasma Bumetanide Dibenzyamide concentration-time data are summarized for all routes of administration in Tables 3-8. Individual animal data are plotted by formulation group on linear and log-linear axes in Figures 1-6.

[00137] Plasma concentration data following IN administration of about 15 mg dose are displayed in Table 3 and plotted in Figures 1A-1B. Bumetanide Dibenzyamide concentrations are variable between dogs during the first 2 hours after dosing with %CV ranging from 75.5 to 105.9%. The variability then decreased until the %CV was 28.5% at 24 hours. Each of the female dogs displayed higher concentrations than the 2 male dogs.

[00138] Table 3: Summary of Plasma Bumetanide Dibenzyamide Concentration-Time Data in

[00139] Dogs Following Administration of a Single Dose of an Intranasal (IN) Formulation

			Time (h)								
			0	0.25	0.5	1.0	2.0	4.0	8.0	12.0	24.0
Route	Dose (mg)	Animal	Bumetanide Dibenzyamide (ng/mL)								
IN	15	2923344	2.63	10.2	12.7	10.9	9.05	6.08	3.47	2.68	2.07
		2960045	1.32	1.40	1.55	2.68	3.73	2.65	1.88	1.68	1.59
		CJCCJW	2.79	3.24	4.14	10.3	41.9	14.2	4.57	3.08	2.43
		CJDCDD	2.79	22.5	29.8	24.7	13.8	7.83	5.55	3.92	3.15

			Time (h)								
			0	0.25	0.5	1.0	2.0	4.0	8.0	12.0	24.0
Route	Dose (mg)	Animal	Bumetanide Dibenzyamide (ng/mL)								
		Mean	2.38	9.34	12.0	12.1	17.1	7.69	3.87	2.84	2.31
		CV%	29.9	102.4	105.9	75.5	99.4	63.0	40.7	32.7	28.5

[00140] Plasma concentration data following SL administration of about 30 mg dose is displayed in Table 4 and plotted in Figures 2A-2B. SL administration provided the lowest concentrations of any of the 6 formulations. Bumetanide Dibenzyamide is absorbed slowly with the first measurable concentration occurring at 0.5 h after dosing in one of the dogs. All dogs have measurable concentrations at 2 hours after dosing. Concentrations are variable through the 24-hour sampling period with %CV ranging from 111.7 to 200%. For the SL route of administration, each of the male dogs displayed higher concentrations than the 2 female dogs.

[00141] Table 4: Summary of Plasma Bumetanide Dibenzyamide Concentration-Time Data in

[00142] Dogs Following Administration of a Single Dose of a Sublingual (SL) Formulation

			Time								
			(h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide Dibenzyamide (ng/mL)								
SL	30	2972702	0.00	0.00	0.00	0.308	0.00	0.00	0.00	0.00	0.00
		2974674	0.00	0.00	0.809	1.53	2.32	1.37	1.10	0.477	0.00
		CJACUH	0.00	0.00	0.00	0.00	1.00	0.686	0.00	0.00	0.00
		CJCCBU	0.00	0.00	0.00	0.505	0.346	0.00	0.00	0.00	0.00
		Mean	0.00	0.00	0.202	0.586	0.917	0.514	0.275	0.119	0.00
		CV%			200.0	113.2	111.7	127.6	200.0	200.0	

[00143] The results following administration of a 15 mg IM dose is illustrated in Table 5 and plotted in Figures 3A-3C. One of the male dogs (animal # 1) displayed very high concentrations, beginning at 0.25 h after dosing, that continued throughout the sampling period, suggesting that the dose may have been inadvertently injected into a vein. The T_{max} for the remaining dogs occurred at 2 to 4 hours indicating slower absorption from the IM injection site.

[00144] Plasma concentration data following PO administration of a 30 mg dose is illustrated in Table 6 and plotted in Figures 4A-4B. The Bumetanide Dibenzyamide T_{max} is at 2 hours after dosing in all 4 animals and the concentrations for all are consistently higher than by any of the other routes of administration. In addition, the variability is lower than for the other formulations and decreased over time, from 51.2% at 0.5 hour to just 9.9% at 24 hours. For the PO route of administration, each of the female dogs displayed higher concentrations than the 2 male dogs.

[00145] The results following SC administration of a 15 mg dose is illustrated in Table 7 and plotted in Figures 5A-5B. Bumetanide Dibenzyamide concentrations were relatively consistent between dogs throughout the 24-hour sampling period but never reached concentrations one might expect following SC administration. One animal (animal #1) displayed a high concentration of 15.2 ng/mL at 1 hour after dosing that appeared to be an outlier based on the concentrations in nearby samples. With the exception of that 1 hour sample the variability in the remainder of the data is relatively low.

[00146] Table 5: Summary of Plasma Bumetanide Dibenzyamide Concentration-Time Data in

[00147] Dogs Following Administration of a Single Dose of an Intramuscular (IM) Formulation

			Time (h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide Dibenzyamide (ng/mL)								
IM	15	2923344	0.00	3.77	5.77	9.58	13.1	10.0	5.43	4.63	2.99
		2960045	0.00	155	112	89.3	70.9	62.4	47.2	36.2	8.23
		CJCCJW	0.00	1.70	2.01	4.06	3.66	4.87	2.54	2.24	1.87
		CJDCDD	0.00	8.59	10.3	16.4	22.7	18.9	8.20	5.54	3.40
		Mean	0.00	42.3	32.5	29.8	27.6	24.0	15.8	12.2	4.12
		CV%		178.0	163.3	133.9	108.4	109.1	132.8	132.4	68.3

[00148] Table 6: Summary of Plasma Bumetanide Dibenzyamide Concentration-Time Data in

[00149] Dogs Following Administration of a Single Dose of an Oral (PO) Formulation

			Time (h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide Dibenzyamide (ng/mL)								
PO	30	2972702	0.00	0.00	7.82	110	158	68.7	21.9	10.5	2.84
		2974674	0.00	0.850	43.7	122	175	56.9	15.7	7.91	2.83
		CJACUH	0.00	3.99	36.8	129	259	95.8	21.5	10.3	2.31
		CJCCBU	0.00	0.480	40.6	227	280	85.3	21.2	8.65	2.86
		Mean	0.00	1.33	32.2	147	218	76.7	20.1	9.34	2.71
		CV%		135.9	51.2	36.7	27.7	22.5	14.6	13.5	9.9

[00150] Plasma concentration data following IR administration of a 30 mg dose is illustrated in Table 8 and plotted in Figures 6A-6B. Mean concentrations of Bumetanide Dibenzyamide are less than 10 ng/mL at all times after dosing. Concentrations are variable through the 24-hour sampling period with %CV ranging from 64.4 to 200%. For the IR route of administration, each of the female dogs once again displayed higher concentrations than the 2 male dogs.

[00151] Table 7: Summary of Plasma Bumetanide Dibenzyamide Concentration-Time Data in

[00152] Dogs Following Administration of a Single Dose of a Subcutaneous (SC) Formulation

			Time (h)								
			0.0 0	0.2 5	0.50	1.0 0	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide Dibenzylamide (ng/mL)								
SC	15	2923344	1.55	1.78	1.81	2.24	3.18	4.15	4.14	3.22	2.90
		2960045	1.39	6.40	2.08	15.2	4.74	6.61	6.67	5.09	3.70
		CJCCJW	1.65	1.94	2.76	3.62	4.69	7.01	4.54	3.35	2.43
		CJDCDD	2.39	2.86	3.42	4.96	7.90	11.3	7.47	5.77	4.30
		Mean	1.75	3.25	2.52	6.51	5.13	7.27	5.71	4.36	3.33
		CV%	25.4	66.5	28.7	90.7	38.7	40.9	28.4	29.2	24.9

[00153] Table 8: Summary of Plasma Bumetanide Dibenzylamide Concentration-Time Data in

[00154] Dogs Following Administration of a Single Dose of an Intrarectal (IR) Formulation

			Time (h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide Dibenzylamide (ng/mL)								
IR	30	2972702	0.00	0.467	1.05	2.07	1.72	1.24	0.674	0.281	0.00
		2974674	0.00	1.03	2.47	4.34	3.63	2.53	0.623	0.322	0.00
		CJACUH	0.00	1.63	3.25	11.6	17.8	12.3	5.93	2.42	1.93
		CJCCBU	0.00	3.18	6.99	9.56	7.64	3.92	1.17	0.383	0.00
		Mean	0.00	1.58	3.44	6.89	7.70	5.00	2.10	0.852	0.483

			Time (h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide Dibenzyamide (ng/mL)								
		CV%		74.2	73.7	64.4	93.2	99.8	122.2	122.9	200.0

[00155] Mean concentration-time profiles for Bumetanide Dibenzyamide are plotted with formulations superimposed in Figures 7A-7B on linear and log-linear axes. Concentrations are substantially higher for PO administration than for any other route. The log-linear plots indicate that Bumetanide Dibenzyamide concentrations tend to decay in parallel after the 8-hour plasma sample.

[00156] Plasma Bumetanide concentration-time data are measured for all routes of administration. There are only 2 plasma samples in any sample from the study with measurable concentrations of Bumetanide. These measurements could be made in animal #7 at 2 and 4 hours after administration of the PO dose formulation and are 0.34 and 0.47 ng/mL, respectively (Table 9).

[00157] Table 9: Summary of Plasma Bumetanide Concentration-Time Data in Dogs Following

[00158] Administration of a Single Dose of an Oral (PO) Formulation

			Time (h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide (ng/mL)								
PO	30	2972702	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

			Time (h)								
			0.00	0.25	0.50	1.00	2.00	4.00	8.00	12.00	24.00
Route	Dose (mg)	Animal	Bumetanide (ng/mL)								
		2974674	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		CJACUH	0.00	0.00	0.00	0.00	0.34	0.47	0.00	0.00	0.00
		CJCCBU	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Mean	0.000	0.000	0.000	0.000	0.086	0.119	0.000	0.000	0.000
		CV%					200.0	200.0			

[00159] Pharmacokinetic Data Analysis

[00160] In another embodiment described herein, pharmacokinetic (PK) parameters were derived using non-compartmental methods employing Phoenix WinNonlin® version 8.2 (Pharsight Corp; St. Louis, MO). The PK parameters defined in Table 10 are calculated by non-compartmental methods using individual animal concentration-time data. The area under the concentration-time curve from zero time (pre-dose) to the last time point is calculated by a combination of linear and logarithmic trapezoidal methods. The linear trapezoidal method is employed for all incremental trapezoids arising from increasing concentrations and the logarithmic trapezoidal method is used for those arising from decreasing concentrations (linear up/log down method). Nominal blood sampling times are used in the analysis. Concentrations of Bumetanide Dibenzyamide and Bumetanide below the lower limits of quantification (LLOQ) of 0.25 ng/mL are treated as zero for the computation of descriptive statistics for construction of mean concentration-time profiles. Individual below quantitation limits (BQL) results are set to zero for all calculations. No animals are excluded from the analysis. In one aspect, non-compartmental pharmacokinetic analysis provides observed or calculated values of C_{max}, T_{max}, and AUC_{last} for all

animals and estimates of AUCinf and CL/F for those animals where a terminal phase half-life ($t_{1/2}$) could be estimated.

[00161] Table 10: Pharmacokinetic Parameter Definitions

C _{max}	Maximum observed plasma concentration
T _{max}	Time of maximum concentration, obtained directly from the observed concentration versus time data
C ₀	Back predicted plasma concentration to time=0 for IV bolus administration
AUC _{inf}	The area under the concentration-time curve from time 0 extrapolated to infinity, calculated as AUC _{last} + C _{last} /λ _z . Where >20% of AUC _{inf} was derived by extrapolation, or where acceptance criteria for λ _z (defined below for $t_{1/2}$) were not met, AUC _{inf} was deemed not reportable.
CL	Clearance after IV dosing calculated as: Dose/AUC _{inf}
V _z	Volume of distribution after IV dosing calculated as: Dose/(AUC _{inf})(λ _z)
$t_{1/2}$	Terminal phase half-life, estimated as $\ln(2)/\lambda_z$, where λ _z is the elimination rate constant and was estimated by linear regression of logarithmically-transformed concentration versus time data (at least 3 measurable timepoints after C _{max}).
F _{abs}	Absolute bioavailability calculated as: $F_{abs} = (AUC_{po}/AUC_{iv}) * (Dose_{iv}/Dose_{po})$

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[00162] Non-compartmental pharmacokinetic parameters for Bumetanide Dibenzyamide after each route of administration are summarized in Tables 11-16. In one aspect, the 15 mg dose level data for IM, IN, and SC dosing suggests that IM provides the highest exposures with C_{max} averaging 48.9 ng/mL and AUC_{inf} of 411 h*ng/mL. In one aspect, SC dosing provides the next highest exposure with mean C_{max} of 9.42 ng/mL and AUC_{inf} of 231 h*ng/mL. IN dosing is comparable to SC displaying a higher C_{max} of 22.0 ng/mL but slightly lower AUC_{inf} of 211 h*ng/mL. In one aspect, the 30 mg dose level data for PO, IR, and SL dosing indicates that PO administration provides by far the highest exposures with C_{max} averaging 218 ng/mL and a mean AUC_{inf} of 814 h*ng/mL. In one aspect, IR dosing provides the next highest exposure with mean C_{max} of 8.44 ng/mL and AUC_{inf} of 55.1 h*ng/mL. In one aspect, SL dosing provides very low and inconsistent exposures with a mean C_{max} of just 1.03 ng/mL and AUC_{last} of only 4.23 h*ng/mL. Half-life could only be estimated in 1 of the 4 dogs following SL administration. As noted above there were only 2 measurable Bumetanide concentrations observed in the study and so no pharmacokinetic analysis was possible.

[00163] Table 11: Summary of Bumetanide Dibenzyamide Pharmacokinetic Parameters in

[00164] Dogs Following Administration of a Single Dose of an Intranasal (IN) Formulation

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
IN	15	2923344	12.7	0.50	94.4	23.3	164	91.5
		2960045	3.73	2.00	47.0	76.1	221	67.7
		CJCCJW	41.9	2.00	165	19.8	234	64.1
		CJDCDD	29.8	0.50	151	16.3	224	66.8

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
		Mean	22.0	1.25	114	33.9	211	72.5
		CV%	77.6	69.3	47.4	83.5	15.1	17.6

[00165] Table 12: Summary of Bumetanide Dibenzyamide Pharmacokinetic Parameters in

[00166] Dogs Following Administration of a Single Dose of a Sublingual (SL) Formulation

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
SL	30	2972702	0.308	1.00	0.0770			
		2974674	2.32	2.00	14.1	5.26	17.7	1690
		CJACUH	1.00	2.00	2.17			
		CJCCBU	0.505	1.00	0.547			
		Mean	1.03	1.50	4.23	5.26	17.7	1690
		CV%	87.7	38.5	157.4			

[00167] Table 13: Summary of Bumetanide Dibenzyamide Pharmacokinetic Parameters in

[00168] Dogs Following Administration of a Single Dose of an Intramuscular (IM) Formulation

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
IM	15	2923344	13.1	2.00	135	18.7	215	69.6
		2960045	155	0.25	926	6.16	999	15.0
		CJCCJW	4.87	4.00	63.0	38.1	166	90.5

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
		CJDCDD	22.7	2.00	202	13.4	268	56.0
		Mean	48.9	2.06	331	19.1	412	57.8
		CV%	145.3	74.3	120.8	71.6	95.5	55.1

[00169] Table 14: Summary of Bumetanide Dibenzylamide Pharmacokinetic Parameters in

[00170] Dogs Following Administration of a Single Dose of an Oral (PO) Formulation

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
PO	30	2972702	158	2.00	675	5.62	698	43.0
		2974674	175	2.00	639	6.79	666	45.0
		CJACUH	259	2.00	893	5.10	910	33.0
		CJCCBU	280	2.00	956	5.89	980	30.6
		Mean	218	2.00	791	5.85	814	37.9
		CV%	27.7	0.0	19.9	12.1	19.1	18.9

[00171] Table 15: Summary of Bumetanide Dibenzylamide Pharmacokinetic Parameters in

[00172] Dogs Following Administration of a Single Dose of a Subcutaneous (SC) Formulation

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
SC	15	2923344	4.15	4.00	79.8	36.2	231	64.8
		2960045	15.2	1.00	129	22.2	247	60.7
		CJCCJW	7.01	4.00	91.3	19.1	158	94.7

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
		CJDCDD	11.3	4.00	153	21.5	286	52.4
		Mean	9.42	3.25	113	24.8	231	68.2
		CV%	51.5	46.2	29.7	31.3	23.2	27.0

[00173] Table 16: Summary of Bumetanide Dibenzylamide Pharmacokinetic Parameters in

[00174] Dogs Following Administration of a Single Dose of an Intranasal (IN) Formulation

Route	Dose (mg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)	CL/F (L/h)
IN	15	2923344	12.7	0.50	94.4	23.3	164	91.5
		2960045	3.73	2.00	47.0	76.1	221	67.7
		CJCCJW	41.9	2.00	165	19.8	234	64.1
		CJDCDD	29.8	0.50	151	16.3	224	66.8
		Mean	22.0	1.25	114	33.9	211	72.5
		CV%	77.6	69.3	47.4	83.5	15.1	17.6

[00175] In one embodiment, the pharmacokinetic profile of Bumetanide Dibenzylamide, when administered as a single oral gavage or as an intravenous dose to rats was evaluated.

[00176] Study Design

[00177] Male Hsd:Sprague Dawley rats were assigned to two groups, and doses were administered as indicated in Table 17. Animals were dosed via oral gavage or intravenous injection once on Day 1 at a volume of 5 mL/kg for Group 1 and 10 mL/kg for Group 2. The vehicle was 40% (v/v) Polyethylene Glycol 400 (PEG400), 20% (v/v) Propylene

Glycol, 5% (v/v) Ethanol, and 35% (v/v) water for Group 1 and the vehicle was 0.5% (w/v) Carboxymethylcellulose (medium viscosity) in reverse osmosis water for Group 2.

[00178] Table 17: Group Assignments and Treatment Information for Phase I

Group	Subgroup	No. of Animals ^{a,b}	Dose Route	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
		Males				
1	1 (Toxicokinetic)	6	Intravenous	10	5	2
2	1 (Toxicokinetic)	6	Oral Gavage	30	10	3
a All animals were dosed on Day 1 of the dosing phase.						
b Phase I animals were used for the Pharmacokinetic profile only.						

[00179] Oral administration was selected because it is the intended route of administration in humans. The dose levels chosen for Phase I were based on tolerability data following intravenous (IV) administration in dogs, allometrically scaled to the rat.

[00180] Plasma Concentration Data

[00181] Blood samples (approximately 0.5 mL) were collected from nonfasted toxicokinetic animals via a jugular vein on Day 1 of the dosing phase, as indicated in Table 18

[00182] Table 18: Blood Collection

Group	Set	Dosing Phase Day	Time Points ^a
1	1st three animals/sex/group	1	5 minutes; 2, 6, and 12 hours postdose

1	2nd three animals/sex/group	1	1, 4, 8, and 24 hours postdose
2	1st three animals/sex/group	1	30 minutes; 2, 6, and 12 hours postdose
2	2nd three animals/sex/group	1	1, 4, 8, and 24 hours postdose
a Blood collection times were approximate.			

[00183] Blood was collected into tubes containing potassium (K₂) EDTA as the anticoagulant. Samples were maintained on wet ice and were centrifuged within 30 minutes of collection. Plasma was harvested and stored on dry ice until placed in a freezer, set to maintain -60 to -80°C, until shipped on dry ice to Origin Bioanalytical Laboratory, Inc. for analysis. Plasma samples were analyzed for Bumetanide Dibenzylamide and Bumetanide content.

[00184] Pharmacokinetic Data Analysis

[00185] Pharmacokinetic parameters were derived using non-compartmental methods employing Phoenix WinNonlin® version 8.1 (Pharsight Corp; St. Louis, MO). The PK parameters defined previously in Table 10 were calculated by non-compartmental methods using composite plasma concentration-time data obtained from 3 rats/sex/time point/dose. The area under the concentration-time curve from zero time (pre-dose) to the last time point was calculated by a combination of linear and logarithmic trapezoidal methods. The linear trapezoidal method was employed for all incremental trapezoids arising from increasing concentrations and the logarithmic trapezoidal method was used for those arising from decreasing concentrations (linear up/log down method). Nominal blood sampling times were used in the analysis. Concentrations of Bumetanide Dibenzylamide below the lower limit of quantification (LLOQ) of 0.25 ng/mL were treated as zero for the computation of descriptive statistics for construction of mean concentration-time profiles. Individual BQL results were set to zero for all calculations. No animals were excluded from the analysis.

[00186] Plasma Bumetanide Dibenzylamide and Bumetanide Concentration-Time Data

[00187] Plasma Bumetanide Dibenzylamide and Bumetanide concentration time data are summarized following IV administration of 10 mg/kg Bumetanide Dibenzylamide in Tables 19-20, respectively. Mean concentration-time profiles are illustrated with analytes overlaid in Figures 8A-8B on linear and log-linear axes. Observed Bumetanide Dibenzylamide concentration averaged 2510 ng/mL at 5 minutes after the IV bolus dose. Measurable concentrations in all 3 animals were observed at 24 hours after dosing. In contrast, Bumetanide concentrations were lower, averaging 53.6 ng/mL at 5 minutes and no animals had measurable concentrations at 24 hours after dosing. Plasma concentration time data was summarized following oral administration of 30 mg/kg Bumetanide Dibenzylamide in **Error! Reference source not found.** 21 and mean concentration-time profiles with analytes overlaid are displayed in **Error! Reference source not found.** The highest mean concentration of Bumetanide Dibenzylamide was 4.56 ng/mL occurring 4 hours after dosing. Bumetanide concentrations were negligible with only 2 rats having measurable concentrations. One was 0.30 ng/mL at 4 hours and the other was 0.28 ng/mL at the 6-hour sampling time.

[00188] Mean plasma Bumetanide Dibenzylamide concentrations plotted in **Error! Reference source not found.** reveal that Bumetanide Dibenzylamide concentrations are considerably lower after oral versus IV bolus dosing. The individual rat plasma concentration-time data used in the analysis are presented in Table 22.

[00189] Table 19: Summary of Plasma Bumetanide Dibenzylamide Concentrations in Male

[00190] Rats Following IV Bolus Administration of 10 mg/kg Bumetanide Dibenzylamide

			Time (h)							
			0.08	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide Dibenzyamide (ng/mL)							
IV	10	R0001	1720		317		203		60.8	
		R0002	3080		477		145		18.6	
		R0003	2720		421		60.8		4.67	
		R0004		993		165		20.0		0.257
		R0005		1190		205		33.1		0.582
		R0006		924		218		87.3		1.95
		N	3	3	3	3	3	3	3	3
		Mean	2510	1040	405	196	136	46.8	28.0	0.930
		CV%	28.1	13.3	20.0	14.1	52.5	76.2	104.3	96.6

[00191] Table 20: Summary of Plasma Bumetanide Concentrations in Male Rats Following IV Bolus Administration of 10 mg/kg Bumetanide Dibenzyamide

			Time (h)							
			0.08	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide (ng/mL)							
IV	10	R0001	44.5		8.33		9.24		3.48	
		R0002	60.3		13.2		11.6		1.29	
		R0003	55.9		10.5		3.09		0.00	

			Time (h)							
			0.08	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide (ng/mL)							
		R0004		26.9		7.36		0.967		0.00
		R0005		24.9		7.07		1.35		0.00
		R0006		25.2		9.01		4.46		0.00
		N	3	3	3	3	3	3	3	3
		Mean	53.6	25.7	10.7	7.81	7.98	2.26	1.59	0.00
		CV%	15.2	4.2	22.9	13.4	55.1	84.8	110.6	

[00192] Table 21: Summary of Plasma Bumetanide Dibenzylamide Concentrations in Male

[00193] Rats Following Oral Administration of 30 mg/kg Bumetanide Dibenzylamide

			Time (h)							
			0.50	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide Dibenzylamide (ng/mL)							
Oral	30	R0101	2.01		4.00		3.99		0.780	
		R0102	1.29		2.88		2.49		0.715	
		R0103	1.67		3.60		4.15		0.731	
		R0104		2.14		3.52		1.57		
		R0105		4.70		5.42		2.57		

			Time (h)							
			0.50	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide Dibenzyamide (ng/mL)							
		R0106		2.45		4.73		3.20		
		N	3	3	3	3	3	3	3	0
		Mean	1.66	3.10	3.49	4.56	3.54	2.45	0.742	
		CV%	21.7	45.1	16.2	21.1	25.8	33.6	4.6	

[00194] Table 22: Summary of Plasma Bumetanide Concentrations in Male Rats Following

[00195] Oral Administration of 30 mg/kg Bumetanide Dibenzyamide

			Time (h)							
			0.50	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide (ng/mL)							
Oral	30	R0101	0.00		0.00		0.00		0.00	
		R0102	0.00		0.00		0.00		0.00	
		R0103	0.00		0.00		0.28		0.00	
		R0104		0.00		0.00		0.00		0.00
		R0105		0.00		0.30		0.00		0.00
		R0106		0.00		0.00		0.00		0.00
		N	3	3	3	3	3	3	3	3

			Time (h)							
			0.50	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Route	Dose (mg/kg)	Animal	Bumetanide (ng/mL)							
		Mean	0.00	0.00	0.00	0.100	0.0920	0.00	0.00	0.00
		CV%				173.2	173.2			

[00196] Pharmacokinetic Parameters for Bumetanide Dibenzyamide and Bumetanide

[00197] Non-compartmental PK parameters for Bumetanide Dibenzyamide and Bumetanide after IV dosing are summarized in Tables 23-24. The back-predicted concentration of Bumetanide Dibenzyamide at time=0 was 2780 ng/mL and the terminal phase half-life was 2.61 hours. CL was high at 2880 mL/h/kg and the Vz was large at 10800 L/kg. Bumetanide concentrations were considerably lower with a Cmax of 53.6 ng/mL occurring at 5 minutes. The half-life was similar to that of Bumetanide Dibenzyamide at 2.91 hours. Non-compartmental PK parameters for Bumetanide Dibenzyamide after oral dosing are summarized in Table 25. The maximum concentration was low at 4.56 ng/mL occurring at 4 hours after dosing. The half-life was identical to the value obtained following IV dosing, at 2.61 hours.

[00198] Table 23: Pharmacokinetic Parameters for Bumetanide Dibenzyamide in Rats following an IV bolus dose of 10 mg/kg Bumetanide Dibenzyamide

Dose Level (mg/kg)	Cmax (ng/mL)	Tmax (h)	C0 (ng/mL)	t½ (h)	AUCinf (h*ng/mL)	CL (mL/h/kg)	Vz (mL/kg)
10	2510	0.08	2720	2.61	3730	2680	10100

[00199] Table 24: Pharmacokinetic Parameters for Bumetanide in Rats following an IV bolus dose of 10 mg/kg Bumetanide Dibenzyamide

Variable	Route	Dose (mg/kg)	C _{max} (ng/mL)	T _{max} (h)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)
Bumetanide	IV	10	53.6	0.08	2.91	112

[00200] Table 25: Pharmacokinetic Parameters for Bumetanide Dibenzyamide in Rats following an Oral dose of 30 mg/kg Bumetanide Dibenzyamide

Variable	Route	Dose (mg/kg)	C _{max} (ng/mL)	T _{max} (h)	t _{1/2} (h)	AUC _{inf} (h*ng/mL)
Bumetanide Dibenzyamide	Oral	30	4.56	4.00	2.61	35.4

[00201] Absolute Bioavailability (F_{abs}) of Bumetanide Dibenzyamide Following Oral and IV Administration to Male Rats

[00202] Absolute bioavailability calculations for Bumetanide Dibenzyamide are summarized in Table 26. The AUC_{inf} for Bumetanide Dibenzyamide following IV bolus dosing at a dose level of 10 mg/kg was 3470 h*ng/mL and the AUC_{inf} following oral dosing of 30 mg/kg was 35.4 h*ng/mL. Adjusting for the doses administered the fraction of Bumetanide Dibenzyamide absorbed was 0.0034 or 0.34%.

[00203] Table 26: Absolute Bioavailability of Bumetanide Dibenzyamide in Rats

Variable	Route	Dose (mg/kg)	AUC _{inf} (h*ng/mL)	F _{abs} (ratio)	F _{abs} (%)
Bumetanide Dibenzyamide	Oral	30	35.4	0.0032	0.32%
	IV	10	3730		

[00204] Though measurable concentrations of Bumetanide Dibenzyamide were observed in rats after IV and oral doses, CL and Vz values after IV administration (2680 mL/h/kg and 10100 mL/kg, respectively) indicate that Bumetanide Dibenzyamide has a high clearance and large volume of distribution in rats. The absolute bioavailability was determined to be very low in the rat, with a fraction absorbed of 0.0032 (0.32%), suggesting the possibility of very high first-pass extraction by the liver. Bumetanide Dibenzyamide Cmax averaged 2510 ng/mL at 5 minutes after administration of the IV dose and 4.56 ng/mL at a Tmax of 4 hours following oral dosing. Measurable concentrations of Bumetanide were observed following IV dosing of Bumetanide Dibenzyamide with an average Cmax of 53.6 ng/mL at 5 minutes after dose administration. The Bumetanide concentrations were below the lower limit of quantification of the bioanalytical method (0.25 ng/mL) in all but 2 samples following oral administration of 30 mg/kg Bumetanide Dibenzyamide. The terminal phase half-lives for Bumetanide Dibenzyamide were identical after each route of administration at 2.61 hours. The half-life of Bumetanide after IV administration of Bumetanide Dibenzyamide was 2.91 hours. CL and Vz values after IV administration were 2680 mL/h/kg and 10100 mL/kg indicating that Bumetanide Dibenzyamide has a high clearance and large volume of distribution in rats.

[00205] Oral Delivery of Unformulated Bumetanide Dibenzyamide In one embodiment, the pharmacokinetic profile and bioavailability of Bumetanide Dibenzyamide, and the formation of bumetanide as a metabolite of Bumetanide Dibenzyamide, following oral dose administration of non-formulated Bumetanide Dibenzyamide was evaluated in Beagle dogs.

[00206] The pharmacokinetic profiles and bioavailability of Bumetanide Dibenzyamide, and the formation of Bumetanide as a metabolite of Bumetanide Dibenzyamide, following oral dose administration was evaluated in in Beagle dogs. The dose level used was about 30 mg of Bumetanide Dibenzyamide. 2 dogs were given the dosage and the Bumetanide Dibenzyamide levels (Table 27) were recorded as illustrated in Figures 11A-11B. Bumetanide levels were not measureable.

[00207] Table 27: Summary of Plasma Bumetanide Dibenzyamide Concentrations in Dogs Following Oral Administration of Non-Formulated 30 mg/kg Bumetanide Dibenzyamide

		Time (h)							
		0.50	1.00	2.00	4.00	6.00	8.00	12.00	24.00
Dose Level (mg/kg)	Animal	Bumetanide Dibenzyamide (ng/mL)							
30	D0001	2.99	34.5	105	149	151	101	40.1	4.48
	D0601	2.17	9.94	50.3	136	153	132	50.5	11.3
	Mean	2.58	22.2	77.7	143	152	117	45.3	7.89
	Min	2.17	9.94	50.3	136	151	101	40.1	4.48
	Max	2.99	34.5	105	149	153	132	50.5	11.3

[00208] Absolute bioavailability calculations for Bumetanide Dibenzyamide are summarized in Table 28. Absolute Bioavailability was determined to be about 5.1%

[00209] Table 28: Absolute Bioavailability of Bumetanide Dibenzyamide in Dogs

Route	Dose Level (mg/kg)	Animal	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h*ng/mL)	AUC _{inf} (h*ng/mL)	t _{1/2} (h)	CL/F (L/h/kg)	V _z /F (L/kg)
Oral	30	D0001	151	6.00	1340	1360	3.61	22.0	115
Oral	30	D0601	153	6.00	1450	1520	4.72	19.7	134
		Mean	152	6.00	1390	1440	4.16	20.8	124
		Min	151	6.00	1340	1360	3.61	19.7	115
		Max	153	6.00	1450	1520	4.72	22.0	134

[00210] It will be apparent to one of ordinary skill in the relevant art that suitable modifications and adaptations to the compositions, formulations, methods, processes, reactions, and applications described herein can be made without departing from the scope of any embodiments or aspects thereof. The compositions and methods provided are exemplary and are not intended to limit the scope of any of the specified embodiments. All of the various embodiments, aspects, and options disclosed herein can be combined in any and all variations or iterations. The scope of the compositions, formulations, methods, and processes described herein include all actual or potential combinations of embodiments, aspects, options, examples, and preferences herein described. The exemplary compositions and formulations described herein may omit any component, substitute any component disclosed herein, or include any component disclosed elsewhere herein. The ratios of the mass of any component of any of the compositions or formulations disclosed herein to the mass of any other component in the formulation or to the total mass of the other components in the formulation are hereby disclosed as if they were expressly disclosed. Should the meaning of any terms in any of the patents or publications incorporated by reference conflict with the meaning of the terms used in this disclosure, the meanings of the terms or phrases in this disclosure are controlling. Furthermore, the foregoing discussion discloses and describes merely exemplary embodiments. All patents and publications cited herein are incorporated by reference herein for the specific teachings thereof. In one embodiment, the concentrations of Bumetanide Dibenzyamide and Bumetanide in monkey plasma (K₂EDTA) by LC-MS/MS was analyzed using a qualified bioanalytical method.

[00211] Study Design

[00212] A pharmacokinetic study was done in cynomolgus monkeys following single oral (nasogastric) gavage, sublingual, and intravenous administration. Briefly, Bumetanide Dibenzyamide and Bumetanide and the respective internal standards, Bumetanide Dibenzyamide-d₅ and Bumetanide-d₅, were extracted from 100 µL of monkey plasma using protein precipitation extraction. Calibration curves, blanks, and analytical QCs were prepared in monkey plasma (K₂EDTA). The extracts were evaporated to dryness, reconstituted, and then analyzed by LC-MS/MS. The calibration range was 0.250 – 250 ng/mL for both analytes.

Analytical runs in this study were evaluated for acceptance based on the criteria listed in Table 29.

[00213] Table 29: Acceptance Criteria for Calibration Curves

Item	Acceptance Criteria
Calibration Curve:	Accuracy within $\pm 15\%$ ($\pm 20\%$ at the LLOQ) $\geq 75\%$ of standards within accuracy criteria
Analytical QCs:	Accuracy within $\pm 20\%$ $\geq 2/3$ of QCs within accuracy criteria $\geq 50\%$ of QCs within accuracy criteria at each level
Dilution QCs:	Accuracy within $\pm 20\%$ $\geq 2/3$ of QCs within accuracy criteria for each dilution scheme

[00214] If the criteria for either the calibration curve or the analytical QCs were not met, the analytical run was rejected. If the dilution QCs for a given dilution scheme did not meet criteria, then all of the study samples associated with that dilution scheme were rejected. No deviations were found during the bioanalytical phase of the study conducted by Origin. Calibration curves were derived from the individual analyte to internal standard peak area ratios using the least squared regression of the ratio versus the nominal concentration of the calibration standard, prepared in duplicate. The regression was then used to back-calculate the concentration of each calibration standard, QC sample and study sample. Quadratic ($1/x^2$) regression was used for Bumetanide Dibenzyamide and Linear ($1/x^2$) regression was used for Bumetanide. Analyst[®] Software Version 1.6.3 (Applied Biosystems-MDS Sciex), operated with Windows[®] (Microsoft), was used for instrument control, data acquisition, and peak integrations. Peak area ratios, standard curve regressions, and sample concentration/accuracy values were calculated with Analyst[®] Software Version 1.6.3. Other results (e.g., mean statistics) were calculated with Excel[®] (Microsoft).

[00215] Pharmacokinetic and Bioavailability Data

[00216] Noncompartmental pharmacokinetic parameters for Bumetanide Dibenzyamide at a 30 mg/kg dose level data for PO administration of a first formulation comprising about 3% Bumetanide Dibenzyamide, Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Propylene Glycol, Polyethylene Glycol and water suggested good bioavailability with both males exhibiting higher concentrations than the females. The C_{max} and AUC_{inf} values for the 30 mg/kg dose for NG (Single Oral Nasogastric Gavage) administration of a second formulation comprising about 1.73 w/w% Bumetanide Dibenzyamide, about 32.4 w/w% Polyoxyl 35 Castor Oil (Kolliphor EL), about 31.32 w/w%), Glyceryl Monolinoleate (Maisine CC), about 31.32 w/w% Soybean Oil, and about 3.24 w/w% Ethanol, which is SEDDS/oral lymphatic targeted formulation, were much higher, with an 8-fold higher C_{max} and a 6-fold higher AUC_{inf}. The concentration-time profiles were different from those observed for the first formulation since Bumetanide Dibenzyamide displayed a later median T_{max} and a shorter half-life. There was no apparent sex difference for the second formulation. The pharmacokinetic parameters for Bumetanide Dibenzyamide following an IV bolus a third formulation –comprising about 0.2% Bumetanide Dibenzyamide, Ethanol, Propylene Glycol, 40% Polyethylene Glycol 400, water, administered at a dose level of 2 mg/kg, were used to provide estimates of the absolute bioavailability of Bumetanide Dibenzyamide after PO dosing and values of the true CL and V_z values in monkeys. The absolute bioavailability (%F_{abs}) calculations for the PO formulations based on C_{max} and AUC_{inf} indicated that the second formulation /SEDDS displayed a 6-fold increase in %F_{abs} than the first formulation (from 2.5% to 15%). There were only a limited number of measurable plasma samples containing bumetanide following PO or IV administration. There were no measurable concentrations of Bumetanide after SL dosing. A fourth formulation comprising the composition B listed in Table 54 was also administered in monkeys. Tables 30-33 summarize plasma Bumetanide Dibenzyamide concentrations in monkeys following administration of the various formulations.

[00217] Table 30: Plasma Bumetanide Dibenzyamide Concentrations in Monkey Following Single Oral (Nasogastric) Gavage of Non-Formulated 30 mg/kg Bumetanide Dibenzyamide (First Formulation).

					Time (h)								
					0.00	0.25	0.50	1.00	2.00	4.00	8.00	24.00	32.00
Form	Route	Dose (mg/kg)	Animal	Sex	Bumetanide Dibenzylamide (ng/mL)								
1	NG	30	23153	M	0.00	0.932	3.97	12.1	157	182	99.9	21.6	9.75
			23154	M	0.00	14.9	16.8	29.7	74.0	51.4	45.1	7.47	6.13
			23155	F	0.00	0.992	1.66	8.08	11.1	9.83	17.5	3.28	0.790
			23156	F	0.00	0.398	1.18	3.01	8.35	23.8	17.0	2.02	1.37
				N	4	4	4	4	4	4	4	4	4
				Mean	0.00	4.31	5.90	13.2	62.6	66.8	44.9	8.59	4.51
				SD	0.00	7.07	7.37	11.6	69.8	78.7	39.0	8.98	4.23
				CV%		164.2	124.8	87.7	111.6	118.0	86.8	104.5	93.9
				Min	0.00	0.398	1.18	3.01	8.35	9.83	17.0	2.02	0.790

		Median	0.00	0.962	2.82	10.1	42.6	37.6	31.3	5.38	3.75
		Max	0.00	14.9	16.8	29.7	157	182	99.9	21.6	9.75

[00218] Table 31: Plasma Bumetanide Dibenzylamide Concentrations in Monkey Following Single Oral (Nasogastric) Gavage of Non-Formulated 30 mg/kg Bumetanide Dibenzylamide (Second Formulation).

					Time (h)								
					0.00	0.25	0.50	1.00	2.00	4.00	8.00	24.00	32.00
Form	Route	Dose (mg/kg)	Animal	Sex	Bumetanide Dibenzylamide (ng/mL)								
3	NG	30	23153	M	0.00	0.250	1.55	7.56	16.8	126	442	26.4	6.41
			23154	M	0.00	0.00	3.16	11.3	82.5	410	785	8.13	2.08
			23155	F	0.00	0.619	4.98	20.2	79.3	140	457	9.30	1.63
			23156	F	0.00	0.261	1.71	7.09	37.8	247	674	10.7	1.62
				N	4	4	4	4	4	4	4	4	4
				Mean	0.00	0.283	2.85	11.5	54.1	231	590	13.6	2.94
				SD	0.00	0.255	1.59	6.07	32.1	131	168	8.58	2.33
				CV%		90.1	55.9	52.6	59.4	56.8	28.5	62.9	79.3
				Min	0.00	0.00	1.55	7.09	16.8	126	442	8.13	1.62
				Median	0.00	0.256	2.44	9.43	58.6	194	566	10.0	1.86
				Max	0.00	0.619	4.98	20.2	82.5	410	785	26.4	6.41

[00219] Table 32: Plasma Bumetanide Dibenzylamide Concentrations in Monkey Following Intravenous Administration of 30 mg/kg Bumetanide Dibenzylamide (Third Formulation).

					Time							
					(h)							
					0.08	0.50	1.00	2.00	4.00	8.00	24.00	32.00
Form	Route	Dose (mg/kg)	Animal	Sex	Bumetanide Dibenzyamide (ng/mL)							
4	IV	2	23153	M	1860	717	538	332	149	51.4	3.95	1.47
			23154	M	1430	757	536	316	87.8	26.6	0.942	0.303
			23155	F	1160	555	472	278	129	27.7	8.10	3.80
			23156	F	1310	626	385	214	89.3	17.2	1.28	0.363
				N	4	4	4	4	4	4	4	4
				Mean	1440	664	483	285	114	30.7	3.57	1.48
				SD	301	90.9	72.0	52.5	30.3	14.6	3.31	1.63
				CV%	20.9	13.7	14.9	18.4	26.6	47.4	92.7	110.1
				Min	1160	555	385	214	87.8	17.2	0.942	0.303
				Median	1370	672	504	297	109	27.2	2.62	0.917
				Max	1860	757	538	332	149	51.4	8.10	3.80

[00220] Table 33: Plasma Bumetanide Dibenzyamide Concentrations in Monkey Following Sublingual Administration of 30 mg/kg Bumetanide Dibenzyamide (Fourth Formulation).

					Time								
					(h)								
					0.00	0.25	0.50	1.0	2.0	4.0	8.0	24.0	32.0
Form	Route	Dose (mg/kg)	Animal	Sex	Bumetanide Dibenzyamide (ng/mL)								
2	SL	5	23153	M	0.00	0.00	0.00	0.00	0.00	0.00	0.380	0.363	0.00
			23154	M	0.00	0.00	0.00	0.00	0.621	0.457	0.629	0.629	0.321

					Time (h)								
					0.00	0.25	0.50	1.0	2.0	4.0	8.0	24.0	32.0
Form	Route	Dose (mg/kg)	Animal	Sex	Bumetanide Dibenzyamide (ng/mL)								
			23155	F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			23156	F	0.00	0.00	0.00	0.00	0.00	0.00	0.632	0.00	0.00
				N	4	4	4	4	4	4	4	4	4
				Mean	0.00	0.00	0.00	0.00	0.155	0.114	0.410	0.248	0.0803
				SD	0.00	0.00	0.00	0.00	0.311	0.229	0.298	0.306	0.161
				CV%					200.0	200.0	72.6	123.5	200.0
				Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				Median	0.00	0.00	0.00	0.00	0.00	0.00	0.505	0.182	0.00
				Max	0.00	0.00	0.00	0.00	0.621	0.457	0.632	0.629	0.321

[00221] EXAMPLES**[00222] Example 1: Synthesis of Bumetanide Dibenzyamide**

[00223] Bumetanide Dibenzyamide was synthesized as described below. Bumetanide (960 mg, 2.6 mmol) was dissolved in dimethylformamide (DMF, 10 mL) and l-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC, 560 mg, 3.6 mmol) was added. After about 10 minutes, 1-hydroxybenzotriazole (HOBt, 392 mg, 2.9 mmol) was added and the solution was stirred for an additional 10 minutes. Dibenzyamine (1 mL, 5.2 mmol) was added and the reaction was stirred for 2 hours, at which time the reaction was complete by LC/MS. The reaction was poured into saturated ammonium chloride (20 mL) and extracted with ethyl acetate (2x100 mL). The ethyl acetate, was washed with saturated sodium bicarbonate, water, and brine, and dried over anhydrous magnesium sulfate. The ethyl acetate was removed under reduced pressure to yield 1.0 g (75%) of N, N-dibenzy 3-

aminosulfonyl-5-butylamino-4-phenoxybenzamide (Bumetanide Dibenzylamide) as white solid. Figure 12 shows the X-ray diffractogram analysis of bumetanide dibenzylamide. Figure 13 shows the DSC analysis of bumetanide dibenzylamide. Figure 14 shows the TGA analysis of bumetanide dibenzylamide. Figure 15 shows the DVS analysis of bumetanide dibenzylamide. Figure 16 shows the PSD analysis of bumetanide dibenzylamide. Figure 17 shows the morphology of Bumetanide Dibenzylamide under 40X magnification.

[00224] Example 2: Oral Capsule

[00225] Solubility Evaluation

[00226] The development of the oral capsule formulation was conducted in two parts. First, the basic solubility of Bumetanide Dibenzylamide in various common solubilizers was determined. Then compositions were constructed to obtain a self-emulsifying drug delivery system (SEDDES) using a mixture of medium chain triglycerides, long chain triglycerides, solubilizers, surfactants, and co-solvents.

[00227] The excipients listed in Table 34 are proposed based on the functional category and prior history of use. The ranges provided are estimates based on the available data obtained during the initial development, prior experience, and literature research. These ranges represent the space in which a suitable self-emulsifying drug delivery system may be obtained in combination with the other excipients in ratios adjusted to total 100% w/w.

[00228] Table 34: Common SEDDES excipients

Class/Uses	Excipient examples	Range (%w/w)	
Non-ionic surfactant/solubilizer, emulsifying agent	Caprylocaproyl polyoxylglycerides (Labrasol ALF and Sopftigen), Lauroyl polyoxylglycerides (Gelucire 44/14 or 48/16), Linoleoyl polyoxylglycerides (Labrafil M2125CS), Oleoyl polyoxylglycerides (Labrafil M1944CS), Stearoyl polyoxylglycerides (Gelucire 50/13), Polyoxyl 35 Castor Oil (Kolliphor EL) or Cremophor RH40 (polyoxyl castor oil), vitamin E TPGS, Span 20 (Sorbitan oleate), Tween 20 or 80 (polysorbate), Poloxamers, Solutol HS-15,	10	45

Oil Phase	Arachis oil, soybean oil, castor oil, corn oil, safflower oil, olive oil, apricot kernel oil, sesame oil, cotton seed oil, sunflower seed oil, palm seed oil, coconut oil, rapeseed oil, peanut oil, peppermint oil, hydrogenated soybean oil, hydrogenated vegetable oils, Maisine 35-1 or Maisine CC or Peceol (glyceryl monolinoleate), Beeswax, oleic acid, soy fatty acids, Vitamin E, Labrafac, Miglyol, Crodamol, Capmuyl, Imwitor, Captex and other medium chain triglycerides (MCTs)	15	65
Co-solvent	Propylene Glycol, Capryol™ 90, Lauroglycol™ 90, Glycerin, Polyethylene glycol, ethanol, tetraglycol	0	15
Antioxidant	alpha tocopherol, ascorbyl palmitate, ascorbic acid, butylated hydroxyanisole or butylated hydroxytoluene	0	0.15

[00229] The solubility of Bumetanide Dibenzylamide at 50°C was determined in multiple solubilizers by visual inspection in common SEDDS excipients. Bumetanide Dibenzylamide was added stepwise to 2 grams of solubilizer that was heated to 50°C and allowed to stir at 800 RPM on a stir plate until the Bumetanide Dibenzylamide no longer dissolved in a 10-minute period. The results are presented in Table 35. Bumetanide Dibenzylamide had the highest solubility in Lauroyl Polyoxyl-32 Glycerides (Gelucire 44/14), Caprylocaproyl Polyoxylglycerides (Labrasol ALF), Sorbitan Ester (Span 80), and Polyoxyl 35 Castor Oil (Kolliphor EL), and thus, these were chosen as candidate solubilizers in SEDDS development.

[00230] Table 35: Bumetanide Dibenzylamide solubility in common SEDDS excipients

Excipient/Solubilizer	Bumetanide Dibenzylamide in solution (mg/mL)
Kolliphor EL (Polyoxyl 35 castor oil)	13.5
Captex 300 (Caprylic/Capric Triglyceride, Medium Chain Triglyceride)	<1*
Labrasol ALF (Caprylocaproyl Polyoxylglycerides)	63
Labrafac Lipophile WL 1349	3

(Medium Chain Triglyceride)	
Drakeol 6VR LT (Mineral Oil)	<1*
Labrafil M 1944CS (Oleoyl polyoxyl 1-6 glycerides)	4.5
Propylene Glycol	2
Soybean Oil	2
Oleyl Alcohol	<1*
Lauroyl Polyoxyl-32 Glycerides (Gelucire 44/14)	106
Sorbitan Ester (Span 80)	250
Oleic Acid	<1*
Polyethylene Glycol 400	8.6

[00231] * = Did not visually go into solution

[00232] Capsule Development and Analysis

[00233] Prototype capsule formulations were developed at a less than 2-gram scale. The solubilizers/surfactants were heated up to 60°C while stirring at ~800RPM using a magnetic stir bar and adding Bumetanide Dibenzylamide until it dissolved. The oil phase was then added to the formulations and stirred until the solution became clear. The solutions were cooled to room temperature while stirring and co-solvent was then added (if applicable). Compositions containing long chain triglycerides and increasing amounts of ethanol as a co-solvent in the range (0 to 4.68%w/w) with the maximum solubility of Bumetanide Dibenzylamide determined to be about 1.8% w/w were investigated as illustrated in Table 37 and Table 38. The comparator compositions are listed in Table 36. For complete solubilization of Bumetanide Dibenzylamide, the minimum amount of required ethanol was determined to be about 3.24% (composition 6).

[00234] Table 36: Prototype capsule compositions

Excipient	Composition 1	Composition 2	Composition 3
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	%w/w	mg/ cap	grams	%w/w	mg/ cap	grams	%w/w	mg/ cap	grams
Bumetanide Dibenzylamide	1.59	10.03	0.0318	1.98	10.00	0.032	1.98	10.00	0.032
Phosphatidylcholine (Phosal 75 SA)	22.00	138.60	0.4400						
Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	70.00	441.00	1.4000				39.72	200.94	0.643
Lauroyl Polyoxyl-32 Glycerides (Gelucire 44/14)				19.39	98.13	0.314			
Sorbitan Ester (Span 80)				37.62	190.31	0.609	25.94	131.25	0.420
Caprylic/Capric Triglyceride (Captex 300)	6.41	40.37	0.1282						
Soybean oil				41.01	207.50	0.664	49.78	251.88	0.806
Total	100.00	630.00	2.00	100.00	505.94	1.62	117.42	505.94	1.90

[00235] Table 37: Prototype capsule compositions

Excipient	Composition 4			Composition 5		
	%w/w	mg/cap	grams	%w/w	mg/cap	grams
Bumetanide Dibenzylamide	1.79	11.25	0.032	1.76	11.06	0.032

Polyoxyl 35 Castor Oil (Kolliphor EL)	33.48	210.94	0.600	32.93	207.46	0.600
Glyceryl Monolinoleate (Maisine CC)	32.37	203.91	0.580	31.83	200.55	0.580
Soybean oil	32.37	203.91	0.580	31.83	200.55	0.580
Ethanol				1.65	10.37	0.030
Total	100.00	630.00	1.79	100.00	630.00	1.82

[00236] Table 38: Prototype capsule compositions

Excipient	Composition 6			Composition 7		
	%w/w	mg/cap	grams	%w/w	mg/cap	grams
Bumetanide Dibenzylamide	1.73	10.89	0.032	1.70	10.72	0.032
Polyoxyl 35 Castor Oil (Kolliphor EL)	32.40	204.10	0.600	31.91	201.06	0.600
Glyceryl Monolinoleate (Maisine CC)	31.32	197.30	0.580	30.85	194.36	0.580
Soybean oil	31.32	197.30	0.580	30.85	194.36	0.580
Ethanol	3.24	20.41	0.060	4.68	29.49	0.088
Total	100.00	630.00	1.85	100.00	630.00	1.88

[00237] To test if the composition created macro-dispersions or micro-dispersions, about 30 mg of formulation was added into 1.0 g of water and lightly swirled. The resultant dispersion was visually characterized. SEDDS (macroemulsions) are typically characterized as an opaque milky white uniform liquid, and SEDDS (microemulsions) are characterized

by a clear, isotropic, transparent solution with opalescence. Compositions 4-6 created a functioning SEDDS for Bumetanide Dibenzyamide. Compositions 4 and 6 were selected for future development work, and the intermediate stability of those compositions was evaluated as described below.

[00238] Intermediate Stability

[00239] To determine the chemical stability over a short-term duration, compositions 4 (Table 37) and 6 (Table 38) were scaled to a 100g batch size, encapsulated and stored in 40°C/75% RH conditions for 2 weeks. The antioxidant, butylated hydroxytoluene (BHT) was added to each composition at 0.03% w/w.

[00240] Stability and Storage Analysis

[00241] The capsules were manually packaged into HDPE bottles and heat sealed. There was no significant change in the appearance of the capsules over the first 2 weeks of the stability study as shown in Table 39.

[00242] Table 39: Appearance Results

Batch	Time Point	Appearance Specification	Results
Composition 6 40°C/75% RH	Initial	Off-white capsule with no visible breaks, leaks or defects on the surface	Conforms
	2 Weeks		Conforms
Composition 4 40°C/75% RH	Initial	Off-white capsule with no visible breaks, leaks or defects on the surface	Conforms
	2 Weeks		Conforms

[00243] The results for assay and related substances are illustrated in Table 40. From these results, it is evident that both drug product lots: compositions 4 and 6, do not produce a significant amount of degradation products when stored at 40°C/75% RH conditions.

[00244] Table 40: Assay and Related Substance results

Formulation	Composition 6		Composition 4	
Peak ID	T=0 wks	T=2 wks	T=0 wks	T=2 wks
UNK RRT 0.803	0.086	0.126	0.081	0.117
UNK RRT 0.861		0.056		
UNK RRT 0.901		0.064		0.063
UNK RRT 0.932		0.106		0.096
Assay RRT 1.0	104.9	101.4	101.9	101.4
UNK RRT 1.064	0.116	0.111	0.121	0.112
UNK RRT 1.082	1.002	1.016	1.045	1.085
UNK RRT 1.095	0.121	0.09	0.13	0.131
UNK RRT 1.112		0.072		
UNK RRT 1.166 (BHT)	2.831	2.819		2.86
UNK RRT 1.184		0.059	2.917	0.057
UNK RRT 1.191			0.051	
UNK RRT 1.224		2.174	0.262	2.14
Total %Area	4.2	6.7	4.6	6.7
Total %Area - Placebo	0.145	2.649	0.378	2.514

[00245] Based on this data, formulation Capsule of composition 6 with about 3.24% Ethanol (shown in Table 38) was selected for use in the animal studies. As shown in Table 41, Bumetanide Dibenzyamide concentration may be adjusted from about 0 to about 1.75 %w/w with corresponding adjustment of Polyoxyl 35 Castor Oil (Kolliphor EL) %w/w while maintaining ratio of all other excipients for a total weight of 100%.

[00246] Table 41: Bumetanide Dibenzyamide Capsule Composition

Excipient	Composition 6
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	mg/cap	%w/w	grams
Bumetanide Dibenzylamide	10.03	1.75	1.750
Polyoxyl 35 Castor Oil (Kolliphor EL)	185.45	32.36	32.365
Glyceryl Monolinoleate (Maisine CC)	179.35	31.30	31.300
Soybean oil	179.35	31.30	31.300
Ethanol	18.62	3.25	3.250
Butylated hydroxytoluene	0.20	0.03	0.035
Total	573.00	100.00	100.00

[00247] Example 3: Nasal Solution

[00248] Solubility Evaluation

[00249] A study was conducted to evaluate the solubility of Bumetanide Dibenzylamide in various solvent/co-solvent systems (Table 43). The solvents were chosen from a group consisting of Caprylocaproyl Polyoxylglycerides (Labrasol ALF), propylene glycol, PEG-400, vitamin E TPGS, ethanol, water, and any combination thereof.

[00250] The excipients listed in Table 42 are proposed based on the functional category and prior history of use. The ranges provided are estimates based on the available data obtained during the initial development, prior experience, and literature research. These ranges represent the space in which a suitable solution for nasal delivery may be obtained in combination with the other excipients in ratios adjusted to total 100% w/w.

[00251] Table 42: Common excipients for nasal solution

Class/Uses	Excipient examples	Range (%w/w)	
Non-ionic surfactant/solu	Caprylocaproyl polyoxylglycerides (Labrasol ALF), Lauroyl polyoxylglycerides (Gelucire 44/14 or 48/16), Linoleoyl	1	80

bilizer, emulsifying agent	polyoxylglycerides (Labrafil M2125CS), Oleoyl polyoxylglycerides (Labrafil M1944CS), Stearoyl polyoxylglycerides (Gelucire 50/13), Kolliphor EL (polyoxyl 35 castor oil), vitamin E TPGS, Span 20, 40, 80 (Sorbitan oleate), Tween 20 or 80 (polysorbate)		
Co-solvent	Propylene Glycol, Capryol™ 90, Lauroglycol™ 90, Glycerin, Polyethylene Glycol, ethanol	0	80
Diluent	Water	5	100

[00252] Based on the solubility data and the chemical properties of Bumetanide Dibenzyamide, a low percentage of water is required to achieve reasonable solubility. Therefore, PEG-400 was selected as the primary diluent.

[00253] Table 43: Solubility Evaluation Experimental Design and Composition Summary

Run	Solvent System (%w/w)					
	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	Propylene Glycol	PEG-400	Vitamin E TPGS	Ethanol	Water
Composition 8	100.00					0.00
Composition 9	50.00					50.00
Composition 10		12.00	80.00			8.00
Composition 11	10.00	12.00	70.00			8.00
Composition 12		20.00	70.00	1.00		9.00
Composition 13	10.00	20.00	57.00	1.00	2.00	10.00

[00254] 50g stock solutions of each solvent system were prepared. The active solutions 3.0 mL of each solvent was aliquoted to separate 8 mL clear glass vials. 30 mg $\pm$ 2 of Bumetanide Dibenzyamide was added to each vial and the samples are mixed on a wrist action shaker for 8 hours. Every 30 minutes the solutions were inspected. If no visible Bumetanide Dibenzyamide was present, an additional 10 mg was added and the mixing was continued. This process was repeated until visible saturation of all solvent systems was

obtained. Prior to analysis, the samples were centrifuged at 5,000 RPM for 3 minutes and filtered through a 0.45µm nylon syringe filter to remove excess solids. The samples were quantitated using an HPLC method with UV absorbance detection. Sample measurement was performed in triplicate, and the average results was used to determine the solubility of Bumetanide Dibenzyamide in each system.

[00255] Comparison of the results indicated that the solubility of the drug substance might be significantly affected by the amount of water in the system (Table 44). While Bumetanide Dibenzyamide readily dissolved in Caprylocaproyl Polyoxylglycerides (Labrasol ALF), up to ~66 mg/mL (Composition 8), the solubility was reduced by slightly less than 10X in 50:50 Caprylocaproyl Polyoxylglycerides (Labrasol ALF), and water. The solubility appears to decrease in correlation to each %w/w increase of water in compositions 11-13. When sufficient solubility was achieved, the composition was evaluated for use as a nasal spray. The solubility of Bumetanide Dibenzyamide in 100% Propylene Glycol (PEG 400) and 100% Caprylocaproyl Polyoxylglycerides (Labrasol ALF), (Composition 8) was also evaluated.

[00256] Table 44: Summary of Solubility Results and Estimated Dose Calculations

Run	µg/mL ¹	Dil. Vol. (mL)	Dil. (mL)	mg/mL ²	75% Sat.	per dose	
						0.4 mL	1.0 mL
Composition 8	796.6	0.3	25.0	66.4	49.8	19.9	49.8
Composition 9	240.9	1.0	25.0	6.0	4.5	1.8	4.5
Composition 10	497.4	1.0	25.0	12.4	9.3	3.7	9.3
Composition 11	832.0	0.5	25.0	41.6	31.2	12.5	31.2
Composition 12	633.8	0.5	25.0	31.7	23.8	9.5	23.8
Composition 13	485.3	0.5	25.0	24.3	18.2	7.3	18.2
100% PEG 400	342.8	1.0	25.0	8.6	6.4	2.6	6.4

¹: measured concentration in the analytical dilution

²: concentration of Bumetanide Dibenzyamide in the solvent system

[00257] Saturated solubility of Bumetanide dibenzyamide in the compositions described in Table 42 are in the range of about 0.6% to about 6.64%. The dose calculations included in Table 42 are based on 75% of the concentration at saturation. This is to reduce the risk of precipitation/crystallization due to environmental fluctuations. Additionally, two dose volumes (0.2/0.5 mL) were selected in accordance with the standard range for nasal delivery. The total dose illustrated is based on the equivalent volume delivered to each nostril, 0.4-1.0 mL total per dose.

[00258] A primary solvent system based on composition-11 was selected for further evaluation. To maximize the amount of water in the formulation an additional set of 3 samples were prepared based on this composition and the effect of the water content from about 10 %w/w to about 20 %w/w was evaluated.

[00259] Effect of Water Content

[00260] The amount of water in the formulation may have a significant impact on the solubility of Bumetanide Dibenzyamide. For this reason, 3 additional small scale formulations (F1-F3) were prepared according to the compositions presented in Table 45. Increasing amounts of PVP and Poloxamer were added to compositions F1-F3 to inhibit rapid precipitation upon dilution in water.

[00261] Table 45: Effect of Water Content – Formulation Compositions

Item No.	Ingredient	% w/w		
		F1	F2	F3
1	Bumetanide Dibenzyamide	Excess	Excess	Excess
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	10.00	10.00	10.00
3	Propylene Glycol	9.15	9.15	9.15
4	PEG-400	62.58	59.58	52.58

5	Polyvinylpyrrolidone (PVP)	3.00	2.50	3.00
6	Poloxamer 407 (P407)	3.00	2.50	3.00
7	Purified Water	10.00	14.00	20.00
	Total	100.00	100.00	100.00

[00262] An excess of Bumetanide Dibenzyamide was added to each formulation while mixing at 40-45°C. The solutions were then cooled to ambient room temperature and mixed for ~4 hours prior to filtering and being evaluated by an assay.

[00263] The solubility data for this evaluation was compared to the results obtained from composition-11 that had a similar composition without PVP and Poloxamer (Table 46). These results support the hypothesis that the solubility of Bumetanide Dibenzyamide decreases non-linearly as the water content increases, or as the water: organic solvent ratio changes.

[00264] Table 46: Results for Effect of Water Content

Formulation	%w/w water	mg/mL in Solution
Composition 11	8	41.6
F1	10	30.4
F2	14	25.3
F3	20	23.9

[00265] The solubility of Bumetanide Dibenzyamide was evaluated and a solvent or solvent system that results in the highest possible concentration (mg/mL) of the composition in solution was identified. The solutions were found to comprise from about 20 mg/mL to about 40 mg/mL of Bumetanide Dibenzyamide. The results show that the optimum composition (bumetanide dibenzyamide concentration of at least 30 mg/mL) may comprise

water in the range from about 0% w/w to about 10% w/w. Two separate compositions were selected for short-term stability analysis.

[00266] Intermediate Stability

[00267] Based on the results of the solubility study, a short-term stability assessment was conducted for two proposed compositions of Bumetanide Dibenzyamide nasal solution. Formulation of the composition of the nasal solution was based on solubility evaluation.

[00268] The concentration of Bumetanide Dibenzyamide in the solution was about 30 mg/mL. The short-term stability assessment was performed over two weeks accelerated storage (40°C/75% RH). Testing was conducted at time zero (T=0) and 2 weeks. An overview of the compositions are illustrated in Tables 47-48.

[00269] Table 47: First Formulation - Nasal Solution 30 mg/mL Composition-A

Item#	Ingredient	%w/w	%w/v	mg/mL
1	Bumetanide Dibenzyamide	2.73	3.00	30.00
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	10.00	11.00	110.00
3	Propylene Glycol	12.05	13.26	132.60
4	Polyethylene Glycol 400	67.22	73.94	739.40
5	Water	8.00	8.80	88.00
	Total	100.00	110.00	1100.00

[00270] Table 48: Second Formulation - Nasal Solution 30 mg/mL Composition-B

Item#	Ingredient	%w/w	%w/v	mg/mL
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1	Bumetanide Dibenzylamide	2.82	3.13	31.27
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	4.65	5.17	51.67
3	Propylene Glycol	13.01	14.44	144.44
4	Polyethylene Glycol 400	67.97	75.45	754.49
5	Polyvinylpyrrolidone (K30)	0.92	1.03	10.25
6	Water	10.62	11.79	117.89
	Total	100.00	111.00	1110.00

[00271] The prepared solutions were filtered through a 0.45 µm nylon filter and packaged into clear glass vials. The samples were stored upright in glass serum vials at 40°C/75%RH for 2 weeks. Analysis was performed using an HPLC method with UV detection.

[00272] There were no observable changes in the samples at the end of the study, and no visible precipitation/particulates. The results (Table 49) demonstrated that the change in assay for a composition was less than the analytical precision ($\pm 2.0\%$).

[00273] Table 49: Nasal Solution Stability – Assay Results

Batch #	% Assay of Bumetanide Dibenzylamide		%Diff
	T=0	T= 2Wks	
Composition A	104.8	103.8	1.0
Composition B	105.0	98.2	6.8

[00274] The data for related compounds are reported as percent area relative to the Bumetanide Dibenzylamide peak in the sample (Table 50). These results did not indicate any significant individual or total amount of impurity at time 0 (T=0) or 2 week accelerated stability.

[00275] Table 50: Related Compounds Results

Impurity (by RRT)	Composition A		Composition B	
	T=0	T= 2Wks	T=0	T= 2Wks
0.67	0.05	<LOQ	<LOQ	<LOQ
0.78	0.17	0.14	0.07	0.07
0.81	0.11	0.15	0.11	0.12
0.86	--	<LOQ	--	<LOQ
1.03	<LOQ	<LOQ	<LOQ	<LOQ
Total Imp %	0.33	0.29	0.18	0.19

-- Not Detected

[00276] Both compositions demonstrated suitable short-term stability as it pertained to the appearance and related compounds. Based on these data composition A was selected for use in the animal studies. For composition A, Bumetanide dibenzylamide concentration may be adjusted from about 20 mg/mL to about 40 mg/mL (2 to 4%) with corresponding range of water content 8 to 10% maintaining ratio of all other excipients for a total weight of 100%.

[00277] Example 5: Rectal Gel and Rectal Paste

[00278] Two separate variations were prepared from the nasal solution solvent system to produce a gel (primary formulation) and paste (alternative formulation). Ranges for excipients as shown in Table 51 are the same as for the nasal solution with additional gelling and thickening agents, and water is limited to about 20%. Since a rectal gel formulation comprises about 20% water, the maximum concentration of Bumetanide Dibenzylamide in a rectal gel is

about 2.4%. Since a rectal paste formulation does not comprise water, the maximum concentration of Bumetanide Dibenzyamide in a rectal paste is about 4.16%.

[00279] Table 51: Common excipients for rectal gel and paste

Class/Uses	Excipient examples	Range (%w/w)	
Non-ionic surfactant/solubilizer, emulsifying agent	Caprylocaproyl polyoxylglycerides (Labrasol ALF), Lauroyl polyoxylglycerides (Gelucire 44/14 or 48/16), Linoleoyl polyoxylglycerides (Labrafil M2125CS), Oleoyl polyoxylglycerides (Labrafil M1944CS), Stearoyl polyoxylglycerides (Gelucire 50/13), Kolliphor EL (polyoxyl 35 castor oil), vitamin E TPGS, Span 20, 40, 80 (Sorbitan oleate), Tween 20 or 80 (polysorbate)	1	80
Co-solvent	Propylene Glycol, Capryol™ 90, Lauroglycol™ 90, Glycerin, Polyethylene Glycol, ethanol	0	80
Diluent	Water	0	20
Gelling and Thickening agent	Carbomer, Pemulen (synthetic polymers of acrylic acid)	0.4	10
	Hydroxypropyl cellulose, carboxymethylcellulose, hydroxyethyl cellulose, ethylcellulose, xylulose (cellulose based polymers)		
	Xanthan gum, gellan gum, guar gum, pectin, gelatin, starch, alginate, tragacanth, bentonite, chitosan, carrageenan, acacia (natural polymers)		
	Polyvinyl alcohol, polyethylene oxide, Poloxamer or Pluronic, polyvinyl pyrrolidone or povidone, Polyethylene glycol 3350 (synthetic polymers)		

[00280] For the rectal gel (Table 52), a separate solution of sodium carboxymethyl cellulose (CMC) was prepared and combined with the non-aqueous phase to produce a clear-to-slightly opaque, viscous gel. The amount of CMC selected was based on prior experience and the resulting gel was concluded to be suitable to support the initial animal studies.

[00281] Table 52: Rectal Gel Formulation Composition

Item#	Ingredient	% w/w	mg/g
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1	Bumetanide Dibenzylamide	0.60	6.00
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	10.00	100.00
3	Propylene Glycol	9.15	91.50
4	Polyethylene Glycol 600	53.84	538.42
5	Polyvinylpyrrolidone (K30)	3.60	36.00
6	Poloxamer 407 (P407)	2.40	24.00
7	Sodium CMC	0.41	4.08
8	Purified Water	20.00	200.00
	Total	100.00	1000.00

[00282] For the initial formulation for the rectal paste, a stock solution of the solvent phase (items 1-6 in Table 53) was prepared. PEG-3350 was added stepwise/quantitatively to this solution until a semi-solid paste was obtained, ~7.5% w/w. The amount of Bumetanide Dibenzylamide in the formulation was then adjusted to obtain the target concentration (6mg/g).

[00283] Table 53: Rectal Paste Formulation Composition

Item#	Ingredient	% w/w	mg/g
1	Bumetanide Dibenzylamide	0.60	6.00
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	10.00	100.00
3	Propylene Glycol	9.15	91.50
4	Polyethylene Glycol 600	66.75	667.50

5	Polyvinylpyrrolidone (K30)	3.60	36.00
6	Poloxamer 407 (P407)	2.40	24.00
7	Polyethylene Glycol 3350	7.50	75.00
	Total	100.00	1000.00

[00284] Intermediate Stability

[00285] The gel and paste formulations were then analysed for short-term stability. A short-term stability assessment was conducted for two proposed formulations of the Bumetanide Dibenzyamide rectal dose 6 mg/mL. This study was performed over two weeks accelerated storage (40°C/75%RH).

[00286] Each formulation batch was prepared by weight and about 50 g was packaged in 5 separate 4 oz jars with PTFE lined caps for stability/storage.

[00287] Stability Storage and Analysis

[00288] The samples were stored upright at 40°C/75%RH for 2 weeks. Analysis was performed using an HPLC method with UV detection. The appearance of the rectal gel was viscous, clear to opaque colorless gel. The appearance of the rectal paste was viscous/flowable white-to-off-white opaque paste.

[00289] There were no observable changes in the appearance of (Table 54) the samples at the end of the study, and no visible precipitation/particulates. Both compositions were determined to be close to 100% of the expected assay of (6 mg/g). But, at 2 weeks, there was a slight decrease in the assay of the rectal paste. Also, the gel formulation exhibited a significant drop in the assay value. However, there was no observed increase in the related compounds. Therefore, it is possible that the change in assay may be due to non-uniformity resulting from precipitation of the Bumetanide Dibenzyamide. Also due to the hygroscopicity of the preparations and absorbed water in the high humidity storage conditions may be the cause of the significant decrease in assay.

[00290] Table 54: Rectal Dose Stability – Results for Assay

Batch #	% Assay of Bumetanide Dibenzyamide		%Diff
	T=0	T= 2Wks	
Composition C	99.8	97.4	2.4
Composition D	98.4	89.1	9.3

[00291] There were no significant changes in the related compounds between T=0 and 2 weeks accelerated stability (Table 55). The only impurity detected above 0.10% at relative retention time (RRT) 0.86, was present in all of the samples, and did not change over the course of the study, so this impurity is not likely to be a degradant. From the results for the short-term stability study, composition C was selected for use in the animal studies.

[00292] Table 55: Rectal Dose Stability – Results for Related Compounds

Impurity (by RRT)	Composition C		Composition D	
	T=0	T= 2Wks	T=0	T= 2Wks
0.80	--	0.05	--	--
0.86	0.35	0.33	0.35	0.33
1.17	0.08	0.07	0.08	0.06
Total Imp	0.43	0.45	0.43	0.39

-- Not Detected

[00293] Example 6: Tablet- Buccal or Sublingual Administration

[00294] Two tablet compositions were developed targeting a dose strength of up to about 30mg Bumetanide Dibenzyamide per tablet or capsule. In some instances, the dose strength is about 10mg Bumetanide Dibenzyamide per tablet. Solubility studies similar to

those done for the oral capsule compositions, as described earlier, were done. The solubility studies identified both drug solubility and wettability to be of concern in aqueous media. Additionally, the oral cavity rarely contains more than about 2ml of saliva available for solubilization/oral disintegration. These issues surrounding oral disintegration and solubilization were accounted for during the formulation process.

[00295] A traditional direct blend tablet complete with a wetting agent and solubilizer was developed. Disintegration was evaluated by continual immersion of the tablets with repeated vertical motion in approximately 700ml of 37°C water in a low form 1000ml beaker until complete disintegration is observed.

[00296] Table 56: Common excipients for dry powder

Class/Uses	Excipient examples	Range (%w/w)	
Diluent/Filler	Ceolus KG 802 (Microcrystalline Cellulose), Mannogem EZ (Spray Dried Mannitol), Lactose, Xylitol, carboxymethylcellulose, starch, calcium phosphate, sucrose, dextrin, maltose, sorbitol, maltodextrin, calcium carbonate, hydroxypropylcellulose, ethylcellulose, hydroxypropyl methylcellulose	50	90
Binder	Polyvinylpyrrolidone, starch, sucrose, dextrin, maltose, sorbitol, polyvinylacetate, copovidone, maltodextrin, acacia, gum agar, carboxymethylcellulose, polymethacrylate	0	10
Disintegrate	Polypasdone XL (Super Disintegrate), microcrystalline cellulose, natural clays, aligns, gums and surfactants, sodium starch glycolate	0	10
Lubricant	Magnesium Stearate, talc, metal stearates, stearic acid, boric acid, sodium chloride, benzoate and acetate sodium or magnesium lauryl sulfate, carbowax, cornstarch, sodium lauryl sulfate	0	2
Substrate/absorbant	Neusilin US2 (Magnesium Aluminometasilicate), Ceolus KG 802 (Microcrystalline Cellulose), dibasic calcium phosphate (Fujicalin), maltodextrin, ordered mesoporous silicates, Aerosil, calcium silicate, natural clays	0	50
Glidant	Cabosil M5P (Fumed Silica), colloidal silica, magnesium stearate, purified talc	0	2
Non-ionic surfactant/solubilizer,	Caprylocaproyl polyoxylglycerides (Labrasol and Sopftigen), Lauroyl polyoxylglycerides (Gelucire 44/14 or 48/16), Linoleoyl polyoxylglycerides (Labrafil M2125CS), Oleoyl polyoxylglycerides	0	35

emulsifying agent, wetting agent	(Labrafil M1944CS), Stearoyl polyoxylglycerides (Gelucire 50/13), Polyoxyl 35 Castor Oil (Kolliphor EL) or Cremophor RH40 (polyoxyl castor oil), vitamin E TPGS, Span 20 (Sorbitan oleate), Tween 20 or 80 (polysorbate), Poloxamers, Solutol HS-15,		
Solubilizer/Fixed Oil	Arachis oil, soybean oil, castor oil, corn oil, safflower oil, olive oil, apricot kernel oil, sesame oil, cotton seed oil, sunflower seed oil, palm seed oil, coconut oil, rapeseed oil, peanut oil, peppermint oil, hydrogenated soybean oil, hydrogenated vegetable oils, Maisine 35-1 or Maisine CC or Peceol (glyceryl monolinoleate), Beeswax, oleic acid, soy fatty acids, Vitamin E, Labrafac, Miglyol, Crodamol, Capmuyol, Imwitor, Captex and other medium chain triglycerides (MCTs)	0	35
Co-solvent	Propylene Glycol, Capryol™ 90, Lauroglycol™ 90, Glycerin, Polyethylene Glycol	0	15

[00297] Dry Powder Blend Development

[00298] A dry powder blend may be compressed into a tablet or filled into a capsule. In one embodiment, the dry powder powder is compressed into a tablet for sublingual administration. The tablet may also be used for buccal administration. The tablet may also be swallowed (via oral administration). In another embodiment, a dry powder filled capsule may only be used for oral administration. Table 56 lists common excipients for dry powder which may be used for compression into tablets or filling capsules. Table 57 illustrates the formulation utilized for a tablet prototype batch A created by direct compression. All items were passed through a sieve screen and blended by appropriate means.

[00299] Table 57: Tablet Prototype Batch A Composition

Item #	Ingredient	% w/w	Dose weight (mg)
1	Bumetanide Dibenzylamide	15.0%	30.0
2	Ceolus KG 802 (Microcrystalline Cellulose)	20.0%	40.0
3	Mannogem EZ (Spray Dried Mannitol)	51.0%	102.0
4	Polyplasdone XL (Super Disintegrate)	7.0%	14.0
5	Poloxamer 407 (Wetting Agent)	3.0%	6.0

6	Citric Acid Monohydrate	1.5%	3.0
7	Cabosil M5P (Fumed Silica)	1.0%	2.0
8	Magnesium Stearate	1.5%	3.0
	Total	100.0%	200.0

[00300] After successful completion of the dry blend tablet formulation, efforts were made to attempt to enhance solubilization of the drug. Upon successful creation of the tablet prototype batch A, the tablet prototype batch B was developed wherein Bumetanide Dibenzylamide solubilizers were incorporated directly into the dry blend tablet formulation. Visual solubility analysis provided knowledge that an approximate ratio of 1:2 Bumetanide Dibenzylamide to solubilizers were needed to dissolve the desired quantity of Bumetanide Dibenzylamide into 2mL of water. The 1:1 ratio of Lauroyl Polyoxyl-32 Glycerides (Gelucire 44/14), to Sorbitan Ester (Span 80), was determined by combined melting point, taste, viscosity, and solubilizing ability. The ratio provided a semi-solid, which may be liquefied upon application of low heat. Utilizing a technique known as hot-melt granulation, the surfactants were liquefied at 60°C followed by addition of the necessary quantity of Bumetanide Dibenzylamide and Poloxamer 407. This mixture was kept under constant stirring to ensure complete even dispersal of ingredients. Continuing under constant heating and mixing, Neusilin US2 (Magnesium Aluminometasilicate) was slowly added to adsorb the viscous mixture onto its particles rough surface. Upon complete adsorption the heat was removed with continued mixing until room temperature was reached. The cooled, hot-melt granulation was then sieved and blended with sieved Polyplasdone XL (Super Disintegrate), Cabosil M5P (fumed silica), and Citric Acid Monohydrate, and Magnesium Stearate. Table 58 illustrates the formulation employed for a sublingual tablet prototype batch B, which was utilized for canine studies.

[00301] Table 58: Sublingual Tablet Prototype Batch B Composition

Item #	Ingredient	% w/w	Dose weight (mg)
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1	Bumetanide Dibenzylamide	7.4%	30.0
2	Gelucire 44/14 (Lauroyl Polyoxyl-32 Glycerides, a Water Dispersible Surfactant)	9.9%	40.0
3	Span 80 (Sorbitan Ester, a Water Dispersible Surfactant)	9.9%	40.0
4	Poloxamer 407 (Wetting Agent)	0.5%	0.8
5	Neusilin US2 (Magnesium Aluminometasilicate)	14.8%	60.0
6	Cabosil M5P (Fumed Silica)	2.0%	8.0
7	Polyplasdone XL (Super Disintegrate)	54.3%	220.0
8	Citric Acid Monohydrate	0.7%	3.0
9	Magnesium Stearate	0.5%	2.0
	Total	100.0%	405.0

[00302] A comparison of density analysis of both batches is provided in Tables 59-60. Visual monitoring of the tableting process revealed no weight fluctuations or die filling issues were present. Sublingual tablet prototype batch B flowdex score of 9mm revealed that the flow of this composition is better than suggested that by the density analysis data. Contrasting this is the flowdex score of 26mm recorded for the sublingual tablet prototype batch A, which indicates that the true dry blend flow may be worse than that suggested by the Carr's index or Hausner ratio. This issue regarding flowability may be remedied upon reduction of Bumetanide Dibenzylamide required for any human targeted dose strength (i.e., 10 mg dose).

[00303] Table 59: Dry Blend Physical Analysis

Sublingual Tablet Prototype Batch A			
Bulk Density	0.344g/mL	Tapped Density	0.472g/mL
Carr's Index	27.0%	Hausner Ratio	1.37
Flowdex Score		26mm	

Prototype Batch B			
Bulk Density	0.298g/mL	Tapped Density	0.355g/mL
Carr's Index	16.0%	Hausner Ratio	1.19
Flowdex Score		9mm	

[00304] Table 60: Dry Blend Physical Analysis Reference Table

Flowability	Carr's Index (%)	Hausner Ratio
Excellent	0-10	1.00-1.11
Good	10-15	1.12-1.18
Fair	16-20	1.19-1.25
Slightly Poor	21-25	1.26-1.34
Poor	26-31	1.35-1.45

[00305] Sublingual Tableting Physical Comparisons

[00306] Due to their relatively small sizes, batches were not compressed by means of rotary tablet compression. Instead, a single station flexi-tab apparatus utilizing a feed shoe that fills a single die by means of gravity was employed. Tablets were purposely made soft with the understanding that packaging will ultimately be comprised of foil-to-foil blisters. Wetting refers to the time needed for blue dyed water to completely permeate through a tablet. Disintegration refers to the time needed for a tablet to be void of a hard core, determined by probing with a thin metal spatula. Table 61 lists the lists the physical properties of the two formulations.

[00307] Table 61: Comparison of Tablet Physical Parameters

Sublingual Tablet Prototype Batch A

Tablet Tooling	Compression Force	Tablet Thickness	Tablet Weight	Tablet Hardness	Wetting / Disintegration
3/8" SC	4kN	3.5mm	201mg	5.6kP	22sec /32sec
Sublingual Tablet Prototype Batch B					
1/2" SC	3.5kN	5.2mm	410mg	3.2kP	29sec/ 96sec

[00308] Intermediate Stability

[00309] The two separate sublingual tablet formulations were monitored over a 2-week time period and monitored by visual appearance, assay, and related compound data across a 2week 40°C/75%RH stability study. Specifics regarding tablet formulation composition, and physical characteristics may be referenced below. Both compositions consisted of tablets containing about 30 mg dosage strengths of Bumetanide Dibenzyamide designed to be delivered to the sublingual region. Tables and procedures listed outline the compositions and preparations utilized for the creation of both stability batches.

[00310] The items for preparation of the sublingual tablet prototype batch B composition are outlined in Table 58. Items#2-4 were heated in an appropriately sized vessel to 60°C under constant stirring. Upon complete liquification of items#2-4, item#1 was added to the vessel continuing constant temperature and mixing for 5-minutes. Item#5 was slowly added to the vessel with continuing constant temperature and mixing. Heat was removed and the granulation were mixed until items were reduced to room temperature. The granulation was sieved through a #40mesh (420 micron) screen. Items#6-7 were sieved through a #40 mesh (420 micron) screen. Item#8 was sieved through a #20mesh (840 micron) screen. The granulation and items#6-8 were added to an appropriately sized vessel. The vessel was about 60% charged with material. A 3D shake mixer (turbula) was used to blend items#1-8 for about 20-minutes. Item#9 was sieved through a #40mesh (420 micron) screen. Item#9 was added to the vessel containing items#1-8 and blended by 3D shake mixer for about 5-minutes. 405mg tablets were created using a 1/2" standard concave tooling with 3.5kN of force.

[00311] The items for preparation of the sublingual tablet prototype batch A composition are outlined in Table 57. Items#1-5 were sieved through a #40mesh (420 micron) screen. Item 6 was sieved through a #20mesh (840 micron) screen. Items 1-6 were added to an appropriately sized vessel. The vessel was about 60% charged with material. A 3D shake mixer (turbula) was used to blend items#1-6 for about 20-minutes. Item#7 was sieved through a #40mesh (420 micron) screen. Item#7 was added to the vessel containing items#1-6, and blended by a 3D shake mixer for 5-minutes. Item#8 was sieved through a #40mesh (420 micron) screen. Item#8 was added to the vessel containing items#1-7 and blended by 3D shake mixer for about 5-minutes. 200mg tablets were created using 3/8" standard concave tooling with 4kN of force.

[00312] Stability Storage and Analysis

[00313] The study was conducted with tablets stored at 40°C/75%RH. Tablets were stored at 30 count per bottle complete with coil, and a 1gram desiccant in heat sealed 60cc HDPE bottles. Tablets were monitored for their respective physical appearance, assay values, and related compounds.

[00314] Samples were stored and tested as per Table 62. Analysis was done at time zero (T=0) and at 2 weeks, wherein the compositions were kept at 40°C/75%RH. The target criteria were 90.0-110.0% of LC.

[00315] Table 62: Stability Testing Criteria

Test	Time Point (weeks)		Target Criteria
	T-0	2wk	
Assay of Bumetanide Dibenzylamide	X	A	90.0-110.0% of LC
Related Compounds	X	A	Report Results > 0.05%
Appearance	X	A	Report Results

X: Time Zero

A: 40°C/75%RH

[00316] The results were recorded over the 2-week stability period monitored. Analysis was broken down by sections into Visual Appearance Results, Assay Results, and Related Compounds Results. All tablets conformed to the appearance specification and there was no significant change in the appearance of the tablets for each batch over the first 2 weeks of the stability study. The appearance results are presented in Table 63. All time points analyzed were well within stability criteria (Tables 64). Assay values appear to have decreased over the stability period. However, due to the lack of increase in related compounds over the same time it may be concluded that the drop in assay values are extremely unlikely to have been caused by degradation. It is important to note that due to the minimal time points analyzed a trend cannot be established.

[00317] Table 63: Appearance Results for Stability Batches

Batch	Time Point	Appearance Specification	Results
Batch A 40°C/75% RH	Initial	Off-white tablet with no visible erosion, discoloration, or defects on the surface	Conforms
	2 Weeks		Conforms
Batch B 40°C/75% RH	Initial	Off-white tablet with no visible erosion, discoloration, or defects on the surface	Conforms
	2 Weeks		Conforms

[00318] Table 64: Sublingual Tablet Assay Stability Results

Formulation	Time Point	Assay
Secondary Prototype Batch#184A-2010	T=0 weeks	99.4
	T=2 weeks	94.2
	T=0 weeks	107.7

Primary Prototype Batch#184A-2009	T=2 weeks	101.3
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[00319] Neither batch monitored over the 2-week stability period accrued any notable increases to related compounds (Table 65). The only related compounds recorded had negligible change over the time analyzed. This provides evidence that all processing methods utilized between both batches had little effect on the chemical stability of the Bumetanide Dibenzylamide, providing confidence that a hot melt granulation technique may be performed without extreme concern of degradation. Thermodynamic results gathered from DSC analysis provides further confidence that temperature exposure up to 60°C is tolerable.

[00320] Table 65: Related Compound Results for Stability Batches

Formulation	Time Point	Total %Area Related Compound	Related Compound @ RRT0.803	Related Compound @ RRT0.861
Secondary	T=0 weeks	0.12	0.08	0.04
Prototype Batch#184A-2010	T=2 weeks	0.13	0.09	0.04
Primary Prototype Batch#184A-2009	T=0 weeks	0.14	0.09	0.05
	T=2 weeks	0.13	0.09	0.04

[00321] Results confirm both formulations analyzed to be stable and acceptable for animal studies within the time-period monitored. The hot melt granulation did not completely solubilize all the Bumetanide Dibenzylamide, instead forming a slurry or suspension. The slurry would have allowed for a multitude of these agglomerates to persist throughout processing, similar to a direct blend technique.

[00322] Remedies to these issues include increasing batch size and milling to obtain a more uniform particle size. Specific to the hot melt granulation procedure, additional surfactants, and solubilizers may be added to completely solubilize the Bumetanide Dibenzylamide. This may result in additional dry substrate to sorb the increased liquid/semi-solid concentration resulting in larger tablets that are suitable for oral administration. The maximum amount of bumetanide dibenzylamide in the dry powder formulation is based on the solubility in the solubilizer reduced by the amount of absorbant and about half the total weight of the dosage form. The sublingual tablet composition B Table 54 utilizes a 1:1 mixture of Gelucire 44/14 and Span 80 as solublizer and Neusilin US2 absorbant. Since the solubility of bumetanide dibenzylamide in the 1:1 mixture was determined to be approximately 500 mg/g and the amount of Neusilin US2 absorbant required was about 15%, the maximum dose for the sublingual tablet composition B Table 54 is up to about 70 mg or about 17.5%. For sublingual administration the tablet size should be minimized but larger tablets and capsules up to about 1 gram are acceptable for oral administration. For oral administration the tablet or capsule may be formulated up to about 175 mg (17.5%) per dose. However, due to the known 3x decrease in dosage strength which will accompany human needs such an increase in excipients may still likely result in a tablet formulation below about 250 mg per dose.

[00323] Example 7:

[00324] Injection Formulation for SC, IM, IV bolus, IV infusion

[00325] The excipients listed in Table 66 are proposed based on the functional category and prior history of use. The ranges provided are estimates based on the available data obtained during the initial development, prior experience and literature research. These ranges represent the space in which a suitable solution for injection may be obtained in combination with the other excipients in ratios adjusted to total 100% w/w.

[00326] Table 66: Common excipients for injection

Class/Uses	Excipient examples	Range (%w/w)
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Non-ionic surfactant/solubilizer, emulsifying agent	Polyoxyl 35 Castor Oil (Kolliphor EL) or Cremophor RH40 (polyoxyl castor oil), Tween 80 (polysorbate), Solutol HS-15	0	65
Water – insoluble Solvent	soybean oil, castor oil, sesame oil, cotton seed oil, safflower seed oil, olive oil, coconut oil, Vitamin E, cholesterol, medium chain triglycerides (MCTs)	0	100
Water soluble Solvent	Propylene Glycol, Glycerin, Polyethylene Glycol 300/400, ethanol, N-Methyl pyrrolidone, dimethylacetamide, dimethyl sulfoxide, benzylalcohol	0	90
Other solubilization agent	Hydroxypropyl- β -cyclodextrin, sulfobutylether- β -cyclodextrin, α -cyclodextrin,	0	30
Phospholipids	Hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol, L- α -dimyristoylphosphatidylcholine, L- α -dimyristoylphosphatidylglycerol, Lecithin, Phosal75A	0	20

[00327] Sample Preparation

[00328] A study was conducted to evaluate the solubility of Bumetanide Dibenzylamide in various solvent/co-solvent systems. Multiple vehicle compositions were investigated, resulting in a clear dosing solution or suspension at the time of the preparation or shortly after the preparation. Vehicles tested included 55 % Polyethylene glycol 400 (PEG-400) in water, 41 % PEG-400, 12 % Ethanol, 47 % water, 45 % PEG-400, 10 % DMSO, 45 % Water, 31 % PEG-400, 31 % Tetraglycol, 15 % Caprylocaproyl Polyoxylglycerides (Labrasol ALF), 23 % water, 30 % PEG-400, 10 % N-Methyl Pyrrolidone, 10 % DMSO, 50 % water, 25 % PEG-400, 10 % DMSO, 20 % Tetraglycol, 45 % water, 20 % Ethanol in water, 20% Hydroxypropyl- β -Cyclodextrin in water, 11% DMSO, 22% PEG-400, 22% Tetraglycol, 44% water, 44% PEG-400, 0.4% Poloxamer-188, 22% N-Methyl Pyrrolidone, 33% water, and 20% PEG-400, 15% Hydroxypropyl- β -Cyclodextrin in water.

[00329] Multiple vehicle compositions were tested, however all compositions tested resulted in a suspension sample either at the time of the preparation or shortly after the preparation. The final concentration of bumetanide dibenzylamide obtained for the above-mentioned vehicles was

calculated at the point of precipitation ranged between 1.25 to 5.56 mg/mL. Unexpectedly, a vehicle composition of 40% PEG-400, 20% Propylene glycol, 5% Ethanol, 35%, water resulted in a clear solution for a period of at least about 3 hours at room temperature. After filtration with a 0.22 μ m PVDF filter, the sample stayed clear for up to 7 days. The compositions selected for animal studies are described in Table 67 and Table 68.

[00330] Table 67: Injection 2.5 mg/mL

Item#	Ingredient	%w/w	%w/v	mg/mL
1	Bumetanide dibenzylamide	0.23	0.25	2.5
2	Propylene Glycol	20	22	220
3	Polyethylene Glycol 400	40	44	440
4	Ethanol	5	5.5	55
5	Water	35	38.5	385
	Total	100.00	111.00	1100.00

[00331] Table 68: Injection 30 mg/mL

Item#	Ingredient	%w/w	%w/v	mg/mL
1	Bumetanide dibenzylamide	2.73	3.00	30.00
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	10.00	11.00	110.00
3	Propylene Glycol	12.05	13.26	132.60
4	Polyethylene Glycol 400	67.22	73.94	739.40
5	Water	8.00	8.80	88.00
	Total	100.00	110.00	1100.00

[00332] For injection compositions, Bumetanide Dibenzyamide concentration may be adjusted from about 20 mg/mL to about 40 mg/mL (2 to 4%) with corresponding range of water content 8 to 20% maintaining ratio of all other excipients for a total weight of 100%. For injection compositions with >20% water content, Bumetanide dibenzyamide concentration is limited to up to about 2.5 mg/mL (0.25%).

[00333] Example 8: Oral Liquid (Solution and Suspension)

[00334] The excipients listed in Table 69 are proposed based on the functional category and prior history of use. The ranges provided are estimates based on the available data obtained during the initial development, prior experience, and literature research. These ranges represent the space in which a suitable solution or suspension for oral delivery may be obtained in combination with the other excipients in ratios adjusted to total 100% w/w.

[00335] Table 69: Common excipients for oral solution and suspension

Class/Uses	Excipient examples	Range (%w/w)	
Non-ionic surfactant/solubilizer, emulsifying agent	Caprylocaproyl polyoxylglycerides (Labrasol ALF and Sopftigen), Lauroyl polyoxylglycerides (Gelucire 44/14 or 48/16), Linoleoyl polyoxylglycerides (Labrafil M2125CS), Oleoyl polyoxylglycerides (Labrafil M1944CS), Stearoyl polyoxylglycerides (Gelucire 50/13), Polyoxyl 35 Castor Oil (Kolliphor EL) or Cremophor RH40 (polyoxyl castor oil), vitamin E TPGS, Span 20 (Sorbitan oleate), Tween 20 or 80 (polysorbate), Poloxamers, Solutol HS-15,	0	80
Water – insoluble Solvent	Arachis oil, soybean oil, castor oil, corn oil, safflower oil, olive oil, apricot kernel oil, sesame oil, cotton seed oil, sunflower seed oil, palm seed oil, coconut oil, rapeseed oil, peanut oil, peppermint oil, hydrogenated soybean oil, hydrogenated vegetable oils, Maisine 35-1 or Maisine CC (glyceryl monolinoleate), Beeswax, oleic acid, soy fatty acids, Vitamin E, Labrafac, Miglyol, Crodamol, Capmuyol, Imwitor, Captex and other medium chain triglycerides (MCTs)	0	80
Water soluble Solvent	Propylene Glycol, Capryol™ 90, Lauroglycol™ 90, Glycerin, Polyethylene Glycol 300/400/600, ethanol, N-Methyl pyrrolidone, tetraglycol, water, dimethylacetamide, dimethyl sulfoxide	0	90

Other solubilization agent	Hydroxypropyl- β -cyclodextrin, sulfobutylether- β -cyclodextrin, α -cyclodextrin		
Phospholipids	Hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol, L- α -dimyristoylphosphatidylcholine, L- α -dimyristoylphosphatidylglycerol		
Thickening agent	Carbomer, Pemulen (synthetic polymers of acrylic acid) Hydroxypropyl cellulose, carboxymethylcellulose, hydroxyethyl cellulose, ethylcellulose, xylulose (cellulose based polymers)	0	10
	Xanthan gum, gellan gum, guar gum, pectin, gelatin, starch, alginate, tragacanth, bentonite, chitosan, carrageenan, acacia (natural polymers)		
	Polyvinyl alcohol, polyethylene oxide, Poloxamer or Pluronic, polyvinyl pyrrolidone or povidone, Polyethylene glycol 3350 (synthetic polymers)		

[00336] Sample Preparation

[00337] Sample was prepared as previously described under sample development for injections under Example 7. In addition, the compositions shown in **Table 47** and **Table 48** (used for nasal administration) are also oral solution formulations for bumetanide dibenzylamide.

[00338] The solution compositions selected for animal studies are described in Table 70 and Table 71.

[00339] Table 70: Oral Solution 2.5 mg/mL

Item#	Ingredient	%w/w	%w/v	mg/mL
1	Bumetanide dibenzylamide	0.23	0.25	2.5
2	Propylene Glycol	20	22	220
3	Polyethylene Glycol 400	40	44	440
4	Ethanol	5	5.5	55
5	Water	35	38.5	385
	Total	100.00	111.00	1100.00

[00340] Table 71: Oral Solution 30 mg/mL

Item#	Ingredient	%w/w	%w/v	mg/mL
1	Bumetanide Dibenzylamide	2.82	3.13	31.27
2	Caprylocaproyl Polyoxylglycerides (Labrasol ALF)	4.65	5.17	51.67
3	Propylene Glycol	13.01	14.44	144.44
4	Polyethylene Glycol 400	67.97	75.45	754.49
5	Polyvinylpyrrolidone (K30)	0.92	1.03	10.25
6	Water	10.62	11.79	117.89
	Total	100.00	111.00	1110.00

[00341] Table 72: Oral Suspension 20 mg/mL

Item#	Ingredient	%w/w	%w/v	mg/mL
1	Bumetanide Dibenzylamide	1.82	2	20
2	Carboxymethylcellulose (medium viscosity)	0.45	0.5	5
3	Water	97.7	107.5	117.89
	Total	100.00	111.00	1110.00

[00342] For oral solution compositions, Bumetanide dibenzylamide concentration may be adjusted from about 20 mg/mL to about 40 mg/mL (2 to 4%) with corresponding range of water content 8 to 20% maintaining ratio of all other excipients for a total weight of 100%.

For compositions with >20% water content, Bumetanide dibenzylamide concentration is limited to up to about 2.5 mg/mL (0.25%). For oral suspension compositions, Bumetanide dibenzylamide concentration may be adjusted up to about 40 mg/mL (4%) with the addition of a wetting agent or surfactant. One embodiment of an oral suspension composition selected for animal studies is shown in Table 72. A suspension intended for oral gavage administration) was 0.5% w/v Carboxymethylcellulose (medium viscosity) in purified water.

[00343] Example 9: Permeability of Test Compounds in Caco-2 Assay

[00344] Compounds of the present disclosure were tested in a gold standard permeability assay. Unexpectedly, the results demonstrate the tested compounds would not be expected to provide improved permeability. Table 73 demonstrates the unpredictable nature of drug product development.

[00345] Table 73: Permeability Assay for Different Compounds

Compound	Mean P_{app} (10^{-6} cm/s)		Efflux Ratio	Mean %Solution Recovery		Rank	
	A to B	B to A		A to B	B to A	P_{app}	Efflux Transporter Substrate
Nadolol	0.0983	ND	-	97.0	ND	Low	-
Metoprolol	19.2	ND	-	94.7	ND	High	-
Digoxin	0.0223	13.1	586	94.7	108.1	Low	Likely
Bumetanide Dibenzyamide unformulated	<0.480	0.396	>0.825	<4.9	25.4	Low	Poor or non-
Bumetanide morpholinoamide unformulated	0.709	31.0	43.8	87.4	99.6	Moderate	Likely
Bumetanide Dibenzyamide SEDDS	<0.332	0.199	>0.600	<58.3	86.3	Low	Poor or non-

Bumetanide morpholinoamide SEDDS	0.787	33.8	42.9	98.2	102.2	Moderate	Likely
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[00346] Table 73': Permeability Assay for Different Compounds

Compound	Mean P_{app} (10^{-6} cm/s)		Efflux Ratio	Mean %Solution Recovery		Rank	
	A to B	B to A		A to B	B to A	P_{app}	Efflux Transporter Substrate
Nadolol	0.0518	ND	-	98.5	ND	Low	-
Metoprolol	15.5	ND	-	95.7	ND	High	-
Digoxin	0.0154	12.3	800	97.4	103.4	Low	Likely
Bumetanide Diethylamide	1.33	42.0	31.6	77.4	88.5	Moderate	Likely
Bumetanide Diethylamide SEDDS	1.48	32.0	21.6	79.3	90.2	Moderate	Likely

[00347] The signal responses of Bumetanide Dibenzylamide in A to B receiver samples were undetectable. The signal responses of Bumetanide Dibenzylamide SEDDS in one A to B receiver sample was undetectable. For the convenience of calculation, 1/300 of the measured peak area ratio (PAR) of the relevant T0 samples were used as the PAR values in these receiver samples. As will be appreciated, using an assumption of zero as the peak area in above conditions, the solution recovery of Bumetanide Dibenzylamide SEDDS was greater than the acceptable cut-off value of 50.0, so the recovery of Bumetanide Dibenzylamide SEDDS was sufficient. The insufficient recovery (%Solution Recovery values < 50.0) observed might complicate the results of Bumetanide Dibenzylamide, therefore, the results generated in this study should be interpreted combining with other in vitro and/or in vivo data as presented herein. The efflux phenomena (efflux ratio >2.00) observed might complicate the prediction of

permeability. Therefore, the permeability results of Bumetanide Morpholinoamide and Bumetanide Morpholinoamide SEDDS generated in this study should be interpreted in combination with the application conditions. The efflux phenomena (efflux ratio >2.00) observed might complicate the prediction of permeability. Therefore, the permeability results of Bumetanide Diethylamide and Bumetanide Diethylamide SEDDS generated in this study should be interpreted in combination with the application conditions. ND means not determined.

Binning Criteria: Low permeability: $P_{app} \leq 0.500 (\times 10^{-6} \text{ cm/s})$; Moderate permeability: $0.500 < P_{app} < 2.50 (\times 10^{-6} \text{ cm/s})$; High permeability: $P_{app} \geq 2.50 (\times 10^{-6} \text{ cm/s})$; and *The binning criteria of permeability are proposed based on Creative Bioarray routine Caco-2 permeability assay conditions (2.00 μM dosing concentration and 120 minutes incubation). The boundaries for low and high permeability binning are equivalent to 50% and 80% of the "calculated Fa" in human; Substrate Potential**; likely: $ERa \geq 2.00$; Poor or non: $ERa < 2.00$.

[00348] Methodology

[00349] Caco-2 Culture

[00350] Caco-2 cells purchased from ATCC were seeded onto polyethylene membranes (PET) in 96-well Corning Insert plates at 1×10^5 cells/ cm^2 , and refreshed medium every 4~5 days until to the 21st to 28th day for confluent cell monolayer formation. Table 74 lists the compound information.

[00351] Table 74: Compound Information

Serial Number	Compound ID FW	Batch No	MW
CPD1	Bumetanide Dibenzylamide 543.68	60495-25-34	543.68
CPD2	Bumetanide Dibenzylamide 433.53	60646-07-34	433.53

CPD3	Bumetanide Dibenzylamide SEDDS 543.68	32920-03-28	543.68
CPD4	Bumetanide Dibenzylamide SEDDS 433.53	32920-04-16	433.53

[00352] Table 74': Compound Information

Serial Number	Compound ID	Batch No	MW
CPD1	Bumetanide Diethylamde	60646-09-18	419.54
CPD2	Bumetanide Diethylamde SEDDS	32920-06-04	419.54

[00353] Transport - Methods

[00354] The transport buffer in the study was HBSS with 10.0 mM HEPES at pH 7.40±0.05. Test compound was tested at 2.00 µM bi-directionally in duplicate. Digoxin was tested at 10.0 µM bi-directionally in duplicate, while nadolol and metoprolol were tested at 2.00 µM in A to B direction in duplicate. Final DMSO concentration was adjusted to less than 1%. The plate was incubated for 2 hours in CO₂ incubator at 37±1°C, with 5% CO₂ at saturated humidity without shaking. And all samples after mixed with acetonitrile containing internal standard were centrifuged at 3200 xg for 10 min. For nadolol and metoprolol, 200 µL supernatant solution was diluted with 600 µL ultra-pure water for LC-MS/MS analysis. For digoxin and test compounds, 200 µL supernatant solution was diluted with 200 µL ultra-pure water for LC-MS/MS analysis. Concentrations of test and control compounds in starting solution, donor solution, and receiver solution were quantified by LC-MS/MS methodologies, using peak area ratio of analyte/internal standard. After transport assay, lucifer yellow rejection assay was applied to determine the Caco-2 cell monolayer integrity.

[00355] Data Analysis

[00356] The apparent permeability coefficient P_{app} (cm/s) was calculated using the equation:

$$P_{app} = (dC_r/dt) \times V_r / (A \times C_0)$$

Where dC_r/dt is the cumulative concentration of compound in the receiver chamber as a function of time ($\mu\text{M/s}$); V_r is the solution volume in the receiver chamber (0.075 mL on the apical side, 0.25 mL on the basolateral side); A is the surface area for the transport, i.e. 0.0804 cm^2 for the area of the monolayer; C_0 is the initial concentration in the donor chamber (μM).

The efflux ratio was calculated using the equation:

$$\text{Efflux Ratio} = P_{app}(\text{BA}) / P_{app}(\text{AB})$$

[00357] Percent recovery was calculated using the equation:

$$\text{\%Solution Recovery} = 100 \times [(V_r \times C_r) + (V_d \times C_d)] / (V_d \times C_0)$$

[00359] Where V_d is the volume in the donor chambers (0.075 mL on the apical side, 0.25 mL on the basolateral side); C_d and C_r are the final concentrations of transport compound in donor and receiver chambers, respectively.

[00360] FIG. 19 represents a tabulated calculation for this Example 9. FIG. 20 is a tabulated depiction of LCMS data for the tested compounds. FIG. 21 is a tabulated depiction of the Lucifer Yellow Rejection Assay for Monolayer Integrity. FIG. 22A, 22B, and 22C likewise are (A) a tabulated calculation of the Permeability of Test Compounds in Caco-2 Assay of Example 9; (B) a tabulated depiction of LCMS data from the Permeability of Test Compounds in Caco-2 Assay of Example 9.; and (C) a tabulated depiction of the Lucifer Yellow Rejection Assay for Monolayer Integrity Test associated with the Permeability of Test Compounds in Caco-2 Assay of Example 9.

[00361] Based on the results of this assay, which is a standard test for predicting permeability of drugs in the intestine, one would not have supported advancing the present disclosure of a SEDDS formulation for the test compounds: Bumetanide Dibenzylamide and Bumetanide Morpholinoamide. Thus, this data supports the unexpected results of the present disclosure.

[00362] Example 10: The SEDDS Formulation increases bioavailability of Bumetanide Amides

[00363] The SEDDS formulation described above increases the bioavailability of Bumetanide Amides including but not limited to Bumetanide Morpholinoamide and Bumetanide Diethylamide.

[00364] Methodology

[00365] SEDDS-formulated and unformulated Bumetanide Morpholinoamide and Bumetanide Diethylamide were orally administered to rats. The experiment was conducted as described previously in regards to Bumetanide Dibenzylamide. Three male and three female rats at each time point for each of the SEDDS and non-SEDDS treatments were used. The female rats showed no concentrations of any drug, at any formulation or time. As this appeared to be experimental error, all female rats were removed from the data.

[00366] Blood concentration of the SEDDS formulated (black triangles) and unformulated (grey circles) Bumetanide Morpholinoamide at 2hrs, 6hrs, and 12hrs post treatment is shown in FIG. 23. An increased concentration of SEDDS formulated Bumetanide Morpholinoamide was observed. However, there was no detectable concentration on blood by 24hrs (data not shown). FIG. 24 shows an exponential fit to the data to predict what the blood concentration values of formulated and unformulated Bumetanide Morpholinoamide might have been at time points prior to 2hrs.

[00367] Blood concentration of the formulated (black triangles) and unformulated (grey circles) Bumetanide Diethylamide at 2hrs, 6hrs, and 12hrs post treatment is shown in FIG. 25. Based on the data recorded, the dosage for both may have been less than an effective amount. Additionally, there was an outlier recorded. However, even if that one outlier is excluded, the average blood concentration of SEDDS formulated is still higher than the blood concentration of unformulated Bumetanide Diethylamide at all time points.

[00368] Based on these observations, SEDDS formulation may be deemed to be an effective method of formulating and administering Bumetanide Amides.

[00369] Those skilled in the art to which the present disclosure pertains may make modifications resulting in other embodiments employing principles of the present disclosure without departing from its spirit or characteristics, particularly upon considering the foregoing teachings. Accordingly, the described embodiments are to be considered in all respects only as illustrative, and not restrictive, and the scope of the present disclosure is, therefore, indicated by the appended claims rather than by the foregoing description or drawings. Consequently, while the present disclosure has been described with reference to particular embodiments, modifications of structure, sequence, materials and the like apparent to those skilled in the art still fall within the scope as claimed.

CLAIMS

That which is claimed is:

1. A pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers.
2. The composition of claim 1, wherein the composition comprises about 2.5 mg/mL to about 42 mg/mL of Bumetanide Dibenzylamide.
3. The composition of claim 1 or 2, wherein the composition comprises about 0.25%w/w to about 15%w/w of Bumetanide Dibenzylamide.
4. The composition of claim 1-3, wherein the composition comprises about 0.1%w/w to about 99.75%w/w of one or more solubilizers.
5. The composition of any one of claims 1–4, wherein the one or more solubilizers are selected from the group consisting of short chain triglycerides, long chain triglycerides, and combinations thereof.
6. The composition of any one of claims 1–5, wherein the one or more solubilizers are selected from the group consisting of Polyoxyl 35 Castor Oil, Glyceryl Monolinoleate, and any combination thereof.

7. The composition of any one of claims 1–6, wherein the one or more solubilizers are selected from the group consisting of Caprylocaproyl Polyoxylglycerides, Phosphatidylcholine, Caprylic/Capric Triglyceride, Lauroyl Polyoxyl-32 Glycerides, Sorbitan Ester, and any combination thereof.
8. The composition of any one of claims 1–7, wherein the one or more solubilizers are selected from the group consisting of Ethanol, Propylene Glycol, Polyethylene Glycol 600, Polyethylene Glycol 3350, Oleyl Alcohol, and any combination thereof.
9. The composition of any one of claims 1–7, wherein the one or more solubilizers are selected from the group consisting of PEG-400, Vitamin E TPGS, and any combination thereof.
10. The composition of any one of claims 1–9, wherein at least one of the one or more solubilizers is soybean oil.
11. The composition of any one of claims 1–10, wherein the composition comprises water.
12. The composition of any one of claims 1–11, wherein the one or more solubilizers are selected from the group consisting of Polyvinylpyrrolidone (K30), Poloxamer 407 (P407), Sodium Carboxymethyl cellulose (CMC), and any combination thereof.

13. The composition of any one of claims 1-12, further comprising at least one super disintegrant.
14. The composition of any one of claims 1-13, further comprising at least one wetting agent.
15. The composition of any one of claims 1-14, further comprising at least one surfactant.
16. The composition of any one of claims 1-15, wherein the composition comprises Ceolus KG (microcrystalline cellulose), Mannogem EZ (spray dried mannitol), Polyplasdone XL (super disintegrant), Poloxamer 407, Lauroyl Polyoxyl-32 Glycerides, Sorbitan Ester, Neusilin US2 (magnesium aluminometasilicate), Citric acid Monohydrate, Cabosil M5P (fumed silica), Magnesium Stearate, or any sub-combination thereof.
17. A pharmaceutical composition of any one of claims 1-16, comprising about 1.79 %w/w of Bumetanide Dibenzylamide, about 33.48 %w/w of Polyoxyl 35 Castor oil, about 32.37 %w/w of Glyceryl Monolinoleate, and about 32.37 %w/w of Soybean oil.
18. A pharmaceutical composition of any one of claims 1-16, comprising about 1.76 %w/w of Bumetanide Dibenzylamide, about 32.93 %w/w of Polyoxyl 35 Castor oil, about 31.83 %w/w of Glyceryl Monolinoleate, about 31.83 %w/w of Soybean oil, and about 10.37 %w/w of Ethanol.

19. A pharmaceutical composition of any one of claims 1-16, comprising about 1.59 %w/w of Bumetanide Dibenzyamide, about 22 %w/w of Phosphatidylcholine, about 70 %w/w of Caprylocaproyl Polyoxylglycerides, and about 6.41 %w/w of Caprylic/Capric Triglyceride.
20. A pharmaceutical composition of any one of claims 1-16, comprising about 1.98 %w/w of Bumetanide Dibenzyamide, about 19.39 %w/w of Lauroyl Polyoxyl-32 Glycerides, about 37.62 %w/w of Sorbitan Ester, and about 41.01 %w/w of Soybean oil.
21. A pharmaceutical composition of any one of claims 1-16, comprising about 1.98 %w/w of Bumetanide Dibenzyamide, about 39.72 %w/w of Caprylocaproyl Polyoxylglycerides, about 25.94 %w/w of Sorbitan Ester, and about 49.78 %w/w of Soybean oil.
22. A pharmaceutical composition of any one of claims 1-16, comprising about 1.73 %w/w of Bumetanide Dibenzyamide, about 32.40 %w/w of Polyoxyl 35 Castor oil, about 31.32 %w/w of Glyceryl Monolinoleate, about 31.32 %w/w of Soybean oil, and about 3.24 %w/w of Ethanol.
23. A pharmaceutical composition of any one of claims 1-16, comprising about 1.7 %w/w of Bumetanide Dibenzyamide, about 31.91 %w/w of Polyoxyl 35 Castor oil, about 30.85 %w/w of Glyceryl Monolinoleate, about 30.85 %w/w of Soybean oil, and about 4.68 %w/w of Ethanol.

24. The pharmaceutical composition of any one of claims 1 – 23, comprising about 10 %w/w to about 100 %w/w of Caprylocaproyl Polyoxylglycerides.
25. The pharmaceutical composition of any one of claims 1 – 24, comprising about 12 %w/w to about 20 %w/w of Propylene Glycol.
26. The pharmaceutical composition of any one of claims 1 – 25 comprising about 57 %w/w to about 80 %w/w of PEG 400.
27. The pharmaceutical composition of any one of claims 1 – 26 comprising about 1% Vitamin E TPGS.
28. A pharmaceutical composition of any one of the claims 1- 27, comprising about 2.73 %w/w of Bumetanide Dibenzylamide, about 10 % w/w of Caprylocaproyl Polyoxylglycerides, about 12.05 %w/w of Propylene Glycol, about 67.22 %w/w of Polyethylene Glycol 400, and about 8 %w/w of Water.
28. A pharmaceutical composition of any one of the claims 1- 27, comprising about 2.82 %w/w of Bumetanide Dibenzylamide, about 4.65 % w/w of Caprylocaproyl Polyoxylglycerides, about 13.01 %w/w of Propylene Glycol, about 67.97 %w/w of Polyethylene Glycol 400, about 0.92 %w/w Polyvinylpyrrolidone (K30), and about 10.62 %w/w of Water.

29. A pharmaceutical composition of any one of the claims 1- 27, comprising about 0.6 %w/w of Bumetanide Dibenzyamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 53.84 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), about 0.41 %w/w Sodium CMC, and about 20 %w/w of water.

30. A pharmaceutical composition of any one of the claims 1- 27, comprising about 0.6 %w/w of Bumetanide Dibenzyamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 66.75 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), and about 7.5 %w/w of Polyethylene Glycol 3350.

31. A pharmaceutical composition of any one of the claims 1- 27, comprising about 15 %w of Bumetanide Dibenzyamide, about 20 %w of Microcrystalline Cellulose, about 51 %w of Spray Dried Mannitol, about 7 %w of Polyplasdone XL (super disintegrate), about 3% w of Poloxamer 407, about 1.5 %w of Citric Acid Monohydrate, about 1.0 %w of Cabosil M5P (fumed silica), and about 1.5 %w of Magnesium Stearate.

32. A pharmaceutical composition of any one of the claims 1- 27, comprising about 7.4 %w of Bumetanide Dibenzyamide, about 9.9 %w of Lauroyl Polyoxyl-32 Glycerides, about 9.9 %w of Sorbitan Ester, about 54.3 %w of Polyplasdone XL (super disintegrate), about 0.5% w of Poloxamer 407, about 0.7 %w of Citric Acid Monohydrate, about 2.0 %w of Cabosil M5P (fumed silica), about 14.8 %w of Neusilin US2 (magnesium aluminometasilicate), and about 0.5 %w of Magnesium Stearate.

33. A pharmaceutical composition comprising of any one of the claims 1- 27, a vehicle composition comprising 55 % Polyethylene Glycol 400 (PEG-400) in water, or 41 % PEG-400, 12 % Ethanol, 47 % water or 45 % PEG-400, 10 % DMSO, 45 % water, or 31 % PEG-400, 31 % Tetraglycol, 15 % Caprylocaproyl Polyoxylglycerides, 23 % water, or 30 % PEG-400, 10 % N-Methyl Pyrrolidone, 10 % DMSO, 50 % water, or 25 % PEG-400, 10 % DMSO, 20 % Tetraglycol, 45 % water, or 20 % Ethanol in water, or 20% Hydroxypropyl- β -Cyclodextrin in water, or 11% DMSO, 22% PEG-400, 22% Tetraglycol, 44% water, or 44% PEG-400, 0.4% Poloxamer-188, 22% N-Methyl Pyrrolidone, 33% water, or 20% PEG-400, 15% Hydroxypropyl- β -Cyclodextrin in water, or 40% PEG-400, 20% Propylene Glycol, 5% Ethanol and 35%, water.

34. A pharmaceutical composition of any one of the claims 1- 27, comprising about 28 g of Bumetanide Dibenzylamide, and about 3 ml of a solvent, wherein 50 g of solvent comprises about 5g of Caprylocaproyl Polyoxylglycerides, about 10g of Propylene Glycol, about 28.5g of PEG-400, about 0.5g of Vitamin E TPGS, about 1g of Ethanol, and about 4.5g of water.

35. A method for treating a patient in need thereof comprising:

administering a pharmaceutical composition comprising Bumetanide Dibenzylamide and one or more solubilizers.

36. The method of claim 35, wherein the pharmaceutical composition comprises about 2.5 mg/mL to about 42 mg/mL of Bumetanide Dibenzylamide.

37. The method of any one of the claims 35-36, wherein the pharmaceutical composition comprises about 0.25%w/w to about 15%w/w of Bumetanide Dibenzylamide.

38. The method of any one of the claims 35-37, wherein the one or more solubilizers comprise short chain triglycerides, long chain triglycerides, or combinations thereof.

39. The method of any one of the claims 35-38, wherein the composition comprises about 0.1%w/w to about 99.75%w/w of one or more solubilizers

40. The method of any one of the claims 35-39, wherein the one or more solubilizers comprise polyoxyl 35 castor oil, Glyceryl Monolinoleate, or any combination thereof.

41. The method of any one of the claims 35-40, wherein the one or more solubilizers comprise Caprylocaproyl Polyoxylglycerides, Phosphatidylcholine, Caprylic/Capric Triglyceride, Lauroyl Polyoxyl-32 Glycerides, Sorbitan Ester or any combination thereof.

42. The method of any one of the claims 35-41, wherein the one or more solubilizers comprise Ethanol, Propylene Glycol, Polyethylene Glycol 600, Polyethylene Glycol 3350, Oleyl Alcohol, or any combination thereof.

43. The method of any one of the claims 35-42, wherein the one or more solubilizers comprise PEG-400, Vitamin E TPGS, or any combination thereof.

44. The method of any one of the claims 35-43, wherein the one or more solubilizers comprises Soybean oil.

45. The method of any one of the claims 35-44, wherein the one or more solubilizers comprises water.

46. The method of any one of the claims 35-45, wherein the one or more solubilizers comprise Polyvinylpyrrolidone (K30), Poloxamer 407 (P407), Sodium Carboxymethyl cellulose (CMC), or any combination thereof.

47. The method of any one of the claims 35-46, wherein the one or more solubilizers comprises at least one super disintegrant.

48. The method of any one of the claims 35-47, wherein the one or more solubilizers comprises at least one wetting agent.

49. The method of any one of the claim 35-48, wherein the one or more solubilizers comprises at least one surfactant.

50. The method of any one of the claim 35-49, wherein the one or more solubilizers comprises Ceolus KG (microcrystalline cellulose), Mannogem EZ (spray dried mannitol), Polyplasdone XL (super disintegrant), Poloxamer 407, lauroyl polyoxyl-32 glycerides, sorbitan ester, Neusilin US2 (magnesium aluminometasilicate), Citric acid Monohydrate, Cabosil M5P (fumed silica), Magnesium Stearate or any combination thereof.

51. The method of claim 35, wherein the pharmaceutical composition comprises about 1.79 %w/w of Bumetanide Dibenzylamide, about 33.48 %w/w of Polyoxyl 35 Castor oil, about 32.37 %w/w of Glyceryl Monolinoleate, and about 32.37 %w/w of Soybean oil.

52. The method of claim 35, wherein the pharmaceutical composition comprises about 1.76 %w/w of Bumetanide Dibenzylamide, about 32.93 %w/w of Polyoxyl 35 Castor oil, about 31.83 %w/w of Glyceryl Monolinoleate, about 31.83 %w/w of Soybean oil, and about 10.37 %w/w of Ethanol.

53. The method of claim 35, wherein the pharmaceutical composition comprises about 1.59 %w/w of Bumetanide Dibenzylamide, about 22 %w/w of Phosphatidylcholine, about 70 %w/w of Caprylocaproyl Polyoxylglycerides, and about 6.41 %w/w of Caprylic/Capric Triglyceride.

54. The method of claim 35, wherein the pharmaceutical composition comprises about 1.98 %w/w of Bumetanide Dibenzylamide, about 19.39 %w/w of Lauroyl Polyoxyl-32 Glycerides, about 37.62 %w/w of Sorbitan Ester, and about 41.01 %w/w of Soybean oil.

55. The method of claim 35, wherein the pharmaceutical composition comprises about 1.98 %w/w of Bumetanide Dibenzylamide, about 39.72 %w/w of Caprylocaproyl Polyoxylglycerides, about 25.94 %w/w of Sorbitan Ester, and about 49.78 %w/w of Soybean oil.

56. The method of claim 35, wherein the pharmaceutical composition comprises about 1.73 %w/w of Bumetanide Dibenzylamide, about 32.40 %w/w of Polyoxyl 35 Castor oil,

about 31.32 %w/w of Glyceryl Monolinoleate, about 31.32 %w/w of Soybean oil, and about 3.24 %w/w of Ethanol.

57. The method of claim 35, wherein the pharmaceutical composition comprises about 1.7 %w/w of Bumetanide Dibenzylamide, about 31.91 %w/w of Polyoxyl 35 Castor oil, about 30.85 %w/w of Glyceryl Monolinoleate, about 30.85 %w/w of Soybean oil, and about 4.68 %w/w of Ethanol.

58. The method of claim 35, wherein the pharmaceutical composition comprises about 10 %w/w to about 100 %w/w of Caprylocaproyl Polyoxylglycerides.

59. The method of claim 35, wherein the pharmaceutical composition comprises about 12 %w/w to about 20 %w/w of Propylene Glycol.

60. The method of claim 35, wherein the pharmaceutical composition comprises about 57 %w/w to about 80 %w/w of PEG 400.

61. The method of claim 35, wherein the pharmaceutical composition comprises about 1% Vitamin E TPGS.

62. The method of claim 35, wherein the pharmaceutical composition comprises about 2.73 %w/w of Bumetanide Dibenzylamide, about 10 % w/w of Caprylocaproyl Polyoxylglycerides, about 12.05 %w/w of Propylene Glycol, about 67.22 %w/w of Polyethylene Glycol 400, and about 8 %w/w of Water.

63. The method of claim 35, wherein the pharmaceutical composition comprises about 2.82 %w/w of Bumetanide Dibenzylamide, about 4.65 % w/w of Caprylocaproyl Polyoxylglycerides, about 13.01 %w/w of Propylene Glycol, about 67.97 %w/w of Polyethylene Glycol 400, about 0.92 %w/w Polyvinylpyrrolidone (K30), and about 10.62 %w/w of Water.

64. The method of claim 35, wherein the pharmaceutical composition comprises about 0.6 %w/w of Bumetanide Dibenzylamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 53.84 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), about 0.41 %w/w Sodium CMC, and about 20 %w/w of water.

65. The method of claim 35, wherein the pharmaceutical composition comprises about 0.6 %w/w of Bumetanide Dibenzylamide, about 10 %w/w of Caprylocaproyl Polyoxylglycerides, about 9.15 %w/w of Propylene Glycol, about 66.75 %w/w of Polyethylene Glycol 400, about 3.6 %w/w of Polyvinylpyrrolidone (K30), about 2.4 %w/w of Poloxamer 407 (P407), and about 7.5 %w/w of Polyethylene Glycol 3350.

66. The method of claim 35, wherein the pharmaceutical composition comprises about 15 %w of Bumetanide Dibenzylamide, about 20 %w of Microcrystalline Cellulose, about 51 %w of Spray Dried Mannitol, about 7 %w of Polyplasdone XL (super disintegrate), about 3% w of Poloxamer 407, about 1.5 %w of Citric Acid Monohydrate, about 1.0 %w of Cabosil M5P (fumed silica), and about 1.5 %w of Magnesium Stearate.

67. The method of claim 35, wherein the pharmaceutical composition comprises up to about 17.5%w of Bumetanide Dibenzylamide, about 9.9 %w of Lauroyl Polyoxyl-32 Glycerides, about 9.9 %w of Sorbitan Ester, about 54.3 %w of Polyplasdone XL (super disintegrate), about 0.5% w of Poloxamer 407, about 0.7 %w of Citric Acid Monohydrate, about 2.0 %w of Cabosil M5P (fumed silica), about 14.8 %w of Neusilin US2 (magnesium aluminometasilicate), and about 0.5 %w of Magnesium Stearate.

68. The method of claim 35, wherein the pharmaceutical composition comprises a vehicle composition comprising 55 Polyethylene Glycol 400 (PEG-400) in water, or 41 % PEG-400, 12 % ethanol, 47 % water or 45 % PEG-400, 10 % DMSO, 45 % water, or 31 % PEG-400, 31 % Tetraglycol, 15 % Caprylocaproyl Polyoxylglycerides, 23 % water, or 30 % PEG-400, 10 % N-Methyl Pyrrolidone, 10 % DMSO, 50 % water, or 25 % PEG-400, 10 % DMSO, 20 % Tetraglycol, 45 % water, or 20 % Ethanol in water, or 20% Hydroxypropyl- β -Cyclodextrin in water, or 11% DMSO, 22% PEG-400, 22% Tetraglycol, 44 % water, or 44 % PEG-400, 0.4 % Poloxamer-188, 22 % N-Methyl pyrrolidone, 33 % water, or 20 % PEG-400, 15 % Hydroxypropyl- β -Cyclodextrin in water, or 40 % PEG-400, 20 % Propylene Glycol, 5% Ethanol, and 35% water.

69. The method of claim 35, wherein the pharmaceutical composition comprises about 28 g of Bumetanide Dibenzylamide, and about 3 ml of a solvent, wherein 50 g of solvent comprises about 5g of Caprylocaproyl Polyoxylglycerides, about 10g of Propylene Glycol, about 28.5g of PEG-400, about 0.5g of Vitamin E TPGS, about 1g of Ethanol, and about 4.5g of water.

70. The method of any one of the claims 35-69,, wherein the pharmaceutical composition is an oral capsule.

71. The method of any one of the claims 35-69,, wherein the pharmaceutical composition is a nasal solution.

72. The method of any one of the claims 35-69,, wherein the pharmaceutical composition is a rectal gel.

73. The method of any one of the claims 35-69,, wherein the pharmaceutical composition is a rectal paste.

74. The method of any one of the claims 35-69,, wherein the pharmaceutical composition is a sublingual tablet.

75. The method of any one of the claims 35-69,, wherein the pharmaceutical composition is an injectable composition.

76. The method of any one of the claims 35-69,, wherein the administration of the patient with the pharmaceutical composition is done orally.

77. The method of any one of the claims 35-69,, wherein the administration of the patient with the pharmaceutical composition is done intranasally.

78. The method of any one of the claims 35-69,, wherein the administration of the patient with the pharmaceutical composition is done intrarectally.

79. The method of any one of the claims 35-69,, wherein the administration of the patient with the pharmaceutical composition is done sublingually.

80. The method of any one of the claims 35-69,, wherein the administration of the patient with the pharmaceutical composition is done subcutaneously.

80. The method of any one of the claims 35-69,, wherein the administration of the patient with the pharmaceutical composition is done intramuscularly.

82. A pharmaceutical composition of any one of the claims 1-34, comprising Bumetanide Dibenzylamide and one or more organic anion transport (OAT) inhibitors.

83. The pharmaceutical composition of claim 82, wherein the one or more OAT inhibitors are competitive antagonists.

84. The pharmaceutical composition of claim 83 or 82, wherein the Bumetanide Dibenzylamide and the one or more OAT inhibitors are formulated with different release profiles.

85. The pharmaceutical composition of claim 84, wherein at least one OAT inhibitor is formulated to be released prior to release of the Bumetanide Dibenzylamide.

86. The pharmaceutical composition of claim 85, wherein at least one OAT inhibitor is formulated to be released prior to the Bumetanide Dibenzyamide achieving maximum plasma concentration ($C_{\max}$) in a patient receiving the composition.
87. The pharmaceutical composition of any one of claims 82 to 86, wherein the one or more OAT inhibitor is selected from the group consisting of probenecid, aspirin, ibuprofen, acetylsalicylic acid, diclofenac, aspartame, and valproic acid.
88. A pharmaceutical composition of any one of the claims 1-34, comprising Bumetanide Dibenzyamide in a self-emulsifying drug delivery system (SED DS).
89. The pharmaceutical composition of claim 88, wherein the SED DS further comprises an isotropic mixture of oils, solubilizers, surfactants, and co-solvents.
90. A pharmaceutical composition comprising Bumetanide Dibenzyamide in an oral lymphatic targeted formulation.
91. A pharmaceutical composition of any one of the claims 1-34, comprising:
- Bumetanide Dibenzyamide,
- Polyoxyl 35 Castor Oil (Kolliphor EL),
- Glyceryl Monolinoleate (Maisine CC),
- Soybean Oil, and

Ethanol.

92. The composition of claim 90 or 91, comprising: about 1 to 2 w/w% Bumetanide Dibenzylamide, about 30 to 35 w/w% Polyoxyl 35 Castor Oil (Kolliphor EL), about 30 to 35 w/w% Glyceryl Monolinoleate (Maisine CC), about 30 to 35 w/w% Soybean Oil, and about 2.5 to 5 w/w% Ethanol.

93. The composition of any one of claims 90 - 92, comprising: 1.7 w/w% Bumetanide Dibenzylamide, about 32.4 w/w% Polyoxyl 35 Castor Oil (Kolliphor EL), about 31.3 w/w% Glyceryl Monolinoleate (Maisine CC), about 31.3 w/w% Soybean Oil, and about 3.2 w/w% Ethanol.

94. A pharmaceutical composition of any one of the claims 1-34, comprising a prodrug of Bumetanide and one or more oral lymphatic targeting excipients.

95. The pharmaceutical composition of claim 94, wherein the prodrug of Bumetanide is an amide prodrug.

96. The pharmaceutical composition of claim 94 or 95, wherein the prodrug of Bumetanide is one or more of Bumetanide Dibenzylamide, Bumetanide Diethylamide, and Bumetanide Morpholinoamide.

97. The pharmaceutical composition of any one of claims 94 - 96, comprising alkoxylated castor oil.

98. The pharmaceutical composition of any one of claims 94 - 96, comprising Polyoxyl 35 Castor Oil (Kolliphor EL).

99. The pharmaceutical composition of any one of claims 94 - 98, comprising one or more of a mono-, di-, and triglyceride.

100. The pharmaceutical composition of any one of claims 94 - 99, comprising Glyceryl Monolinoleate (Maisine CC).

101. The pharmaceutical composition of any one of claims 94 - 100, comprising a fixed oil.

102. The pharmaceutical composition of any one of claims 94 - 101, comprising soybean oil.

103. The pharmaceutical composition of any one of claims 94 - 102, comprising a water soluble solvent.

104. The pharmaceutical composition of any one of claims 94 - 103, comprising ethanol.

105. The method of any one of the claims 35 - 80, wherein the pharmaceutical composition comprises up to 17.5 %w of Bumetanide Dibenzylamide, about 20 %w solublizer, and about 15 %w absorbant.

106. A method of treating epilepsy comprising administering a compound or composition as defined in any one of claims 1- 105 to a patient in need thereof.

107. A method of treating a seizure disorder comprising administering a compound or composition as defined in any one of claims 1- 105 to a patient in need thereof.

108. A method of treating Alzheimer's Disease comprising administering a compound or composition as defined in any one of claims 1- 105 to a patient in need thereof.