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## DRY BLEND FORMULATION OF TETRAHYDROBIOPTERIN

### CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the priority of U.S. Provisional Application Nos. 61/554,665, filed Nov. 2, 2011; and 61/622,417, filed Apr. 10, 2012; the disclosure of each of which is incorporated herein by reference in its entirety.

### BACKGROUND OF THE DISCLOSURE

#### 1. Field of the Disclosure

This disclosure is generally directed to dry blend, powder formulations and dosage forms of tetrahydrobiopterin. In particular, provided is a dry blending process for tetrahydrobiopterin (i.e., BH4 dihydrochloride), the powder of which can be stably packaged in sachets, stable capsule dosage forms containing a pullulan-free capsule shell, or dissolved into a stable solution and stored in hermetically and non-hermetically sealed containers.

#### 2. Background

Tetrahydrobiopterin (also referred to as BH4 or sapropterin) is a biogenic amine of the naturally occurring pterin family and is a cofactor for a number of different enzymes, including phenylalanine hydroxylase (PAH), tyrosine 3-hydroxylase, tryptophan 5-hydroxylase, and nitric oxide synthase (NOS). Accordingly, BH4 is involved in the synthesis of the amino acids phenylalanine, tyrosine and tryptophan, and the neurotransmitters dopamine and serotonin. Moreover, BH4 is essential for NOS-catalyzed oxidation of L-arginine to L-citrulline and nitric oxide. Pterins are present in physiological fluids and tissues in reduced and oxidized forms, but only the 5,6,7,8-tetrahydrobiopterin is biologically active. More specifically, it is the 6R enantiomer of BH4 that is known to be the biologically active enantiomer. For a detailed review of the synthesis of and disorders associated with BH4, see Blau et al., "Disorders of tetrahydrobiopterin and related biogenic amines" in C. R. Scriver et al., eds., *The Metabolic and Molecular Bases of Inherited Disease*, 8<sup>th</sup> ed., pp. 1275-1776, McGraw-Hill (New York, 2001).

Tetrahydrobiopterin is unstable, readily undergoes aerobic oxidation at room temperature, and has a shelf-life of less than 8 hours at room temperature in aqueous solutions. Due to the instability of BH4, most tetrahydrobiopterin products available on the market need to be specially packaged or kept frozen. The instability of such BH4 compositions is undesirable, and significant degradation resulting from improper storage could hinder therapy of patients. One example of a stable BH4 tablet composition is disclosed in WO 2006/055511, which is incorporated herein by reference in its entirety. Such a tablet composition is sold under the trade-name KUVAN®. There is a need for additional formulations of BH4 suitable for pharmaceutical use.

### SUMMARY

Provided herein is a stable dry blend formulation of tetrahydrobiopterin (BH4) or a BH4-related compound, stable dosages in the form of capsule dosages or dry powder sachet dosages, and therapeutic methods using such dosage forms.

In one aspect of the disclosure, provided herein is a stable dry blend formation of BH4 or a BH4-related compound. In one embodiment, the formulation comprises a dry blend of BH4 or a BH4-related compound, a flavor enhancer, a sweetener, and one or more fillers wherein the components are

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blended together. In certain embodiments, the blending comprises blending the fillers with the BH4 or BH4 compound and flavor enhancer in a blender to achieve a adequate mixture, further blending a portion of the blended mixture with acesulfame potassium or sucralose, a flavoring agent, and ascorbic acid and thereafter passing that mixture through a suitable sieve, and lastly blending the second mixture with the remainder of the first mixture until the blend is homogenous.

In certain embodiments, the BH4 or a BH4-related compound is (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin dihydrochloride (i.e., "BH4 dihydrochloride" or "sapropterin dihydrochloride").

In certain embodiments for example, the fillers are isomalt, lactitol, maltitol, mannitol, sorbitol, xylitol, sucrose, fructose, or combinations thereof.

In certain embodiments, for example, the dry blend formulation also contains a sweetener wherein the sweetener is acesulfame potassium, isomalt, Magna Sweet, maltitol, mannitol, sorbitol, sucralose, xylitol, alitmae, neohesperidin dihydrochalcone, trehalose, tagatose, neotame, saccharin and salts thereof, stevioside, erythritol, isomaltulose, polydextrose, luo han guo, monatin, cyclamate, osladine, sucrose, fructose, or glucose or combinations thereof.

In certain embodiments, for example, the flavor enhancer is anhydrous citric acid, citric acid monohydrate, malic acid, tartic acid, sodium citrate, potassium citrate monohydrate, potassium citrate anhydrous, or sodium potassium tartarate, ascorbic acid, sodium ascorbate, or combinations thereof.

In certain embodiments, for example, the flavoring agent is a cherry, grape, orange, pink lemonade, raspberry, grape, lemon, orange, strawberry, tutti-frutti, tangerine, apple, watermelon, pineapple, banana, peach, kiwi, mango, mixed berry, raspberry lemonade, wild blackberry, blue raspberry, citrus, blueberry, lime, lemon lime, grapefruit, pomegranate, pear, or plum flavors, bubble gum, or combinations thereof.

In certain embodiments, for example, the sieve is a 20 mesh sieve.

In another embodiment, the dry blend formulation is dissolved in an aqueous solution, flushed with an inert gas, and hermetically sealed wherein the active solution remains stable.

In another aspect of the disclosure, stable BH4 or BH4-related compounds can optionally include one or more other therapeutic agents suitable for the condition to be treated. In one embodiment, the other therapeutic agents are selected from folates, including but not limited to folate precursors, folic acids, and folate derivatives, e.g., folinic acid (leucovorin); vitamins, such as vitamin C (ascorbic acid), vitamin B2 (riboflavin), and vitamin B12; neurotransmitter precursors, such as L-dopa, carbidopa, and serotonin; 5-hydroxytryptophan; arginine; and combinations thereof.

In another aspect of the disclosure, provided herein are therapeutic methods using the stable dosage forms described herein. The stable dosage forms are useful for treating, ameliorating, or preventing any BH4-responsive conditions or disorders, e.g., metabolic disorders involving amino acid metabolism. In one embodiment, the stable dosage forms are used to treat subjects exhibiting elevated phenylalanine levels or decreased tyrosine levels, e.g., subjects suffering from hyperphenylalanemia, mild phenylketonuria (PKU), or classic severe PKU. In another embodiment, the stable dosages are used to treat subjects suffering from conditions or disorders that would benefit from enhancement of nitric oxide synthase activity, including, but not limited to, vascular diseases, ischemic or inflammatory diseases, diabetes, and insulin resistance. The total dose of BH4 or BH4-related compound required can be administered in multiple doses or in a