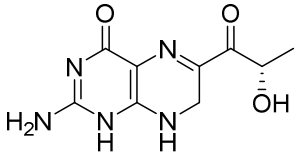
POLYMORPHS OF SEPIAPTERIN AND SALTS THEREOF

BACKGROUND OF THE INVENTION

Sepiapterin is a naturally occurring precursor of tetrahydrobiopterin (BH4), a naturally occurring essential cofactor of critical intracellular enzymes to include but not limited to phenylalanine hydroxylase (PAH) (Kaufman, 1958), tyrosine hydroxylase (TH) (Nagatsu et al, 1964), tryptophan hydroxylase (TPH) (Ichiyama et al, 1970), nitric oxide synthases (NOS) (Kwon et al, 1989), (Mayer et al, 1991) and alkylglycerol monooxygenase (AGMO) (Tietz et al, 1964). Rapid conversion of sepiapterin to BH4 favoring accumulation of BH4 occurs via a two-step reduction in the salvage pathway for BH4 synthesis (Sawabe, 2008). A synthetic form of BH4 (e.g., sapropterin dihydrochloride) is used as a therapy for diseases associated with high plasma phenylalanine, such as phenylketonuria (PKU). PKU is an inborn error of metabolism caused predominantly by mutations in the PAH gene. BH4 was also tested as a therapy for various central nervous symptoms associated with PKU and other diseases, but demonstrated limited effect, presumably due to the inability of BH4 to effectively cross the blood brain barrier (Klaiman et al, 2013; Grant et al, 2015).

Recent work has suggested that, compared with BH4, peripherally administered sepiapterin possesses greater permeability through membranes and as a result, can more readily access liver, kidney, and brain cells. It is reported that sepiapterin is rapidly converted into BH4 intracellularly via the tetrahydrobiopterin-salvage pathway, thereby elevating liver, kidney, and brain BH4 levels (Sawabe, 2008). As a result, sepiapterin may serve as a useful therapeutic for diseases associated with low intracellular BH4 levels or with dysfunction of various BH4 dependent metabolic pathways.

Sepiapterin herein is the *S*-enantiomer and has the formula (I):



(I)

It is known that sepiapterin has limited stability in solutions. Furthermore, certain forms of solid sepiapterin degrade under oxidative conditions even at room temperature and in the presence of light. Accordingly, there exists an unmet need for stable solid-state forms of sepiapterin.

BRIEF SUMMARY

The disclosure provides a solid form of sepiapterin free base, wherein the solid form comprises an amorphous form of sepiapterin free base, a single polymorph form of sepiapterin free base, a mixture of polymorph forms of sepiapterin free base, a salt of sepiapterin or a mixture of salts of sepiapterin, or a combination thereof, with the proviso that the solid form is not a pure polymorph of Form A or Form E sepiapterin free base.

The disclosure also provides crystalline polymorph forms of salts of sepiapterin, or a mixture thereof. As an example, the disclosure provides a crystalline polymorph form of sepiapterin hydrochloride salt.

It has now been surprisingly found that under certain conditions, new crystalline forms of sepiapterin free base and acid salts are formed, which have advantageous utilities and properties. The disclosure thus provides methods for preparing the various polymorphic forms.

The disclosure further provides pharmaceutical compositions comprising one or more of these polymorphic forms.

In one aspect, the present disclosure refers to a Crystalline Form B of sepiapterin free base having peaks at diffraction angle 2θ (°) of 8.4°±0.5, 14.9°±0.5, 16.9°±0.5, 25.4°±0.5, and 34.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In an example, the disclosure features a crystalline form sepiapterin having at least one peak at diffraction angle 2θ (°) of 8.4°±0.5, 16.9°±0.5, or 25.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 8.4°±0.5, 16.9°±0.5, and 25.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 14.9°±0.5, or 34.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 8.4°±0.5, 14.9°±0.5, 16.9°±0.5, 25.4°±0.5, and 34.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In some examples, the crystalline form of sepiapterin has the X-ray powder diffraction spectrum as shown in FIG. 1. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 195°C in differential scanning calorimetry (DSC) profile.

In one aspect, the present disclosure refers to a Crystalline Form C of sepiapterin free base having peaks at diffraction angle 2θ (°) of 5.7°±0.5, 7.8°±0.5, 9.1°±0.5, 11.5°±0.5, and 25.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In an example, the disclosure features crystalline form sepiapterin having at least one peak at diffraction angle 2θ (°) of 5.7°±0.5, 7.8°±0.5, or 25.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 5.7°±0.5, 7.8°±0.5, and 25.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 9.1°±0.5, 11.5°±0.5, 15.3°±0.5, 16.0°±0.5, 20.1°±0.5, or 26.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 5.7°±0.5, 7.8°±0.5, 9.1°±0.5, 11.5°±0.5, 15.3°±0.5, 16.0°±0.5, 20.1°±0.5, 25.4°±0.5, and 26.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In some examples, the crystalline form of sepiapterin has the X-ray powder diffraction spectrum as shown in FIG. 2. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 58 °C, 102 °C, 130 °C, 156.5 °C, or 168°C in differential scanning calorimetry (DSC) profile. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 58 °C, 102 °C, 130 °C, 156.5 °C, and 168°C in differential scanning calorimetry (DSC) profile.

In one aspect, the present disclosure refers to a Crystalline Form D of sepiapterin free base having peaks at diffraction angle 2θ (°) of 8.9°±0.5, 10.3°±0.5, 10.9°±0.5, 26.0°±0.5, and 26.8°±0.5, as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In an example, the disclosure features a crystalline form sepiapterin having at least one peak at diffraction angle 2θ (°) of 8.9°±0.5, 10.3°±0.5, or 26.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 8.9°±0.5, 10.3°±0.5, and 26.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray

diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 10.9°±0.5, 17.8°±0.5, 24.9°±0.5, 26.7°±0.5, 26.8°±0.5, or 28.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 8.9°±0.5, 10.3°±0.5, 10.9°±0.5, 17.8°±0.5, 24.9°±0.5, 26.0°±0.5, 26.7°±0.5, 26.8°±0.5, and 28.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In some examples, the crystalline form of sepiapterin has the X-ray powder diffraction spectrum as shown in FIG. 3. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 43 °C, 66 °C, or 233°C in differential scanning calorimetry (DSC) profile. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 43 °C, 66 °C, and 233°C in differential scanning calorimetry (DSC) profile.

In one aspect, the present disclosure refers to a Crystalline Form F of sepiapterin free base having peaks at diffraction angle 2θ (°) of 9.7°±0.5, 10.2°±0.5, and 11.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In an example, the disclosure features a crystalline form sepiapterin having at least one peak at diffraction angle 2θ (°) of 9.7°±0.5, 10.2°±0.5, or 11.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 9.7°±0.5, 10.2°±0.5, and 11.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 14.0°±0.5, 14.6°±0.5, 19.9°±0.5, 22.2°±0.5, 25.3°±0.5, or 32.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry. In some examples, the crystalline form of sepiapterin has at least one peak at diffraction angle 2θ (°) of 9.7°±0.5, 10.2°±0.5, 11.3°±0.5, 14.0°±0.5, 14.6°±0.5, 19.9°±0.5, 22.2°±0.5, 25.3°±0.5, and 32.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In some examples, the crystalline form of sepiapterin has the X-ray powder diffraction spectrum as shown in FIG. 4. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 113 °C or 196 °C in differential scanning calorimetry (DSC) profile. In some examples, the crystalline form of sepiapterin has an endothermic onset at about 113 °C and 196 °C in differential scanning calorimetry (DSC) profile.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the crystalline Form F of sepiapterin free base as disclosed herein and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a method of preparing a liquid formulation comprising dispersing crystalline Form F of sepiapterin free base as disclosed herein in a liquid.

In one aspect, the present disclosure refers to a Crystalline Form G of sepiapterin free base having peaks at diffraction angle 2θ (°) of 10.0°±0.5, 10.6°±0.5, 15.3°±0.5, 24.4°±0.5, 25.7°±0.5, and 26.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In an example, the disclosure features a composition including any of the foregoing crystalline forms of sepiapterin, or combinations thereof. In some examples of the composition, the crystalline form of sepiapterin of any one of claims 1 to 27, is present in an amount of at least 90 percent by weight of the composition.

In an example, the disclosure features a pharmaceutical composition including any of the foregoing crystalline forms of sepiapterin. In some examples, the crystalline form of sepiapterin is formulated as particles between 50 µm and 250 µm in size (e.g., less than 100 µm in size).

In an example, the disclosure features a method for preparing a crystalline form of sepiapterin including preparing a slurry of a first crystalline form of sepiapterin in water, acetone/water, isopropanol/isopropyl acetate, or tetrahydrofuran/n-hexane, isolating the solids from the slurry, and drying the solids. In some examples, the slurry of the first crystalline form of sepiapterin is stirred at 25-75°

C for 6-72 hours. In some examples, the solids are dried at 20-30 °C for 6-24 hours. In some examples, the solids are dried at 40-60 °C for 5-10 hours. In some examples, the solids are dried at atmospheric pressure. In some examples, the solids are dried under vacuum.

In one aspect, the present disclosure refers to a method for preparing a crystalline form of sepiapterin free base comprising preparing a slurry of a first crystalline form of sepiapterin in water, acetone/water, isopropanol/isopropyl acetate, or tetrahydrofuran/n-hexane, isolating crystalline Form D of sepiapterin free base from the slurry, and drying the crystalline Form D.

In an example, the disclosure features a salt of sepiapterin. In some examples, the salt of sepiapterin is the methansulfonate salt, the nicotinate salt, the p-toluenesulfonate salt, the benzenesulfonate, the phosphate salt, the malonate salt, the tartrate salt, the gentisate salt, the fumarate salt, the glycolate salt, the acetate salt, the sulfate salt, or the hydrochloride salt.

In an example, the disclosure features a crystalline form of a salt of sepiapterin, wherein the crystalline form of a salt of sepiapterin is:

(a) a crystalline form of the methanesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.8°±0.5, 23.5°±0.5, and/or 29.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(b) a crystalline form of the methanesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 21.7°±0.5, 26.0°±0.5, and/or 28.9°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry

(c) a crystalline form of the nicotinate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 9.5°±0.5, 9.9°±0.5, and/or 24.5°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(d) a crystalline form of the p-toluenesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.5°±0.5, 15.1°±0.5, and/or 23.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(e) a crystalline form of the benzenesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.5°±0.5, 14.8°±0.5, and/or 19.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(f) a crystalline form of the phosphate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 16.6°±0.5, 22.2°±0.5, and/or 25.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(g) a crystalline form of the malonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.9°±0.5, 22.7°±0.5, and/or 23.8°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(h) a crystalline form of the tartrate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.3°±0.5, 14.2°±0.5, and/or 21.8°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(i) a crystalline form of the gentisate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.1°±0.5, 8.7°±0.5, and/or 26.7°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(j) a crystalline form of the fumarate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 11.3°±0.5, 24.0°±0.5, and/or 28.2°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(k) a crystalline form of the glycolate salt of sepiapterinhaving at least one peak at diffraction angle 2θ (°) of 7.6°±0.5, 10.7°±0.5, and/or 24.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(l) a crystalline form of the acetate salt of sepiapterinhaving at least one peak at diffraction angle 2θ (°) of 6.2°±0.5, 12.0°±0.5, and/or 18.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(m) a crystalline form of the sulfate salt of sepiapterinhaving at least one peak at diffraction angle 2θ (°) of 5.1°±0.5, 7.8°±0.5, and/or 23.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry; or

(n) a crystalline form of the sulfate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.8°±0.5, 8.8°±0.5, and/or 24.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In one aspect, the present disclosure refers to a crystalline form of a salt of sepiapterin, wherein the crystalline form of a salt of sepiapterin is selected from the group consisting of:

(a) a crystalline form of the methanesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.9°±0.5, 23.5°±0.5, and 29.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(b) a crystalline form of the methanesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 21.7°±0.5, 26.1°±0.5, and 28.9°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry

(c) a crystalline form of the nicotinate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 9.5°±0.5, 9.9°±0.5, and 24.5°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(d) a crystalline form of the p-toluenesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.5°±0.5, 15.1°±0.5, and 23.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(e) a crystalline form of the benzenesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.5°±0.5, 14.8°±0.5, and 19.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(f) a crystalline form of the phosphate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 16.6°±0.5, 22.2°±0.5, and 25.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(g) a crystalline form of the malonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.9°±0.5, 22.7°±0.5, and 23.8°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(h) a crystalline form of the tartrate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.4°±0.5, 14.2°±0.5, and 21.8°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;

(i) a crystalline form of the gentisate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.1°±0.5, 8.7°±0.5, and 26.7°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(j) a crystalline form of the fumarate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 11.4°±0.5, 24.0°±0.5, and 28.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(k) a crystalline form of the glycolate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.6°±0.5, 10.7°±0.5, and 24.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(l) a crystalline form of the acetate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.2°±0.5, 12.0°±0.5, and 18.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(m) a crystalline form of the sulfate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 5.1°±0.5, 7.8°±0.5, and 23.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry; and
(n) a crystalline form of the sulfate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.9°±0.5, 8.8°±0.5, and 24.2°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

In an example, the disclosure features a crystalline form of the hydrochloride salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.8°±0.5, 12.9°±0.5, and/or 26.2°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry

In an example, the disclosure features a composition including any of the foregoing crystalline forms of a salt of sepiapterin. In some examples, the crystalline form of the salt of sepiapterin is present in at least 90 percent by weight.

In an example, the disclosure features a pharmaceutical composition including any of the foregoing crystalline forms of a salt of sepiapterin and a pharmaceutically acceptable carrier. In some examples, the crystalline form of sepiapterin is formulated as particles less than 100 μm in size.

In another example, the disclosure features a method for treating a BH4 related disorder in a patient in need thereof, the method comprising administering to the patient an effective amount of any of the foregoing crystalline forms of sepiapterin or pharmaceutical compositions. In some examples, the BH4-related disorder is a disease associated with low intracellular BH4 levels or with dysfunction of various BH4 dependent metabolic pathways including, but not limited to, primary tetrahydrobiopterin deficiency, GTPCH deficiency, 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency, DHPR deficiency, sepiapterin reductase deficiency, dopamine responsive dystonia, Segawa Syndrome, tyrosine hydroxylase deficiency, phenylketonuria, DNAJC12 deficiency, Parkinson's Disease, depression due to Parkinson’s Disease, impulsivity in Parkinson’s patients, major depression, Autism spectrum, ADHD, schizophrenia, Bipolar disorder, cerebral ischemia, restless leg syndrome, Obsessive Compulsive Disorder, anxiety, aggression in Alzheimer’s disease, cerebrovascular disorders, gastroparesis, spasm after subarachnoidal hemorrhage, myocarditis, coronary vasospasm, cardiac hypertrophy, arteriosclerosis, hypertension, thrombosis, infections, endotoxin shock, hepatic cirrhosis, hypertrophic pyloric stenosis, gastric mucosal injury, pulmonary hypertension, renal dysfunction, impotence, and hypoglycemia. Thus, the various forms of sepiapterin in accordance with the present disclosure can be administered to a patient in an effective amount to obtain a treatment or amelioration of the disease or dysfunction. In one aspect, the present disclosure refers to use of crystalline Form F of sepiapterin free base as disclosed herein in the manufacture of a medicament for treating primary tetrahydrobiopterin deficiency in a patient in need thereof.

In another example, the disclosure features a method of increasing BH4, serotonin, and/or dopamine levels (e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%,

500%, 1000% or more) in a subject in need thereof, the method comprising administering to the patient an effective amount of any of the foregoing crystalline forms of sepiapterin or pharmaceutical compositions.

In another example, the disclosure features a method of decreasing phenylalanine levels (e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 1000% or more) in a subject in need thereof, the method comprising administering to the patient an effective amount of any of the foregoing crystalline forms of sepiapterin or a pharmaceutical compositions. In one aspect, the present disclosure refers to use of crystalline Form F of sepiapterin free base as disclosed herein in the manufacture of a medicament for decreasing phenylalanine levels in a patient in need thereof.

In another example, the disclosure features a method of increasing the activity (e.g., at least 55%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 1000% or more) of phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase, nitric oxide synthase, and/or alkylglycerol monooxygenase in a subject, the method comprising administering to the patient an effective amount of any of the foregoing crystalline forms of sepiapterin or a pharmaceutical compositions.

In another example, the disclosure features a method of treating phenylketonuria in a subject in need thereof, the method comprising administering to the patient an effective amount of any of the foregoing crystalline forms of sepiapterin or pharmaceutical compositions. In one aspect, the present disclosure refers to use of crystalline Form F of sepiapterin free base as disclosed herein in the manufacture of a medicament for treating phenylketonuria in a patient in need thereof.

Definitions

In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”; (iii) the terms “comprising” and “including” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) the terms “about” and “approximately” may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (v) where ranges are provided, endpoints are included.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some examples, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal.

As used herein, the term “BH4 related disorder,” refers to any disease or disorder that may derive a therapeutic benefit from modulation (e.g., inhibition) of the level of BH4, e.g., phenylketonuria.

By “determining the level of a protein” is meant the detection of a protein, or an mRNA encoding the protein, by methods known in the art either directly or indirectly. “Directly determining” means performing a process (e.g., performing an assay or test on a sample or “analyzing a sample” as that term is defined herein) to obtain the physical entity or value. “Indirectly determining” refers to receiving the physical entity or value from another party or source (e.g., a third party laboratory that directly acquired the physical entity or value). Methods to measure protein level generally include, but are not limited to, western blotting, immunoblotting, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, surface plasmon resonance, chemiluminescence,

CLAIMS:

1. Crystalline Form B of sepiapterin free base having peaks at diffraction angle 2θ (°) of 8.4°±0.5, 14.9°±0.5, 16.9°±0.5, 25.4°±0.5, and 34.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

2. Crystalline Form B of sepiapterin free base of claim 1 having the X-ray powder diffraction spectrum as shown in FIG. 1.

3. Crystalline Form B of sepiapterin free base of claim 1 having an endotherm at about 195 °C in differential scanning calorimetry (DSC) profile.

4. Crystalline Form C of sepiapterin free base having peaks at diffraction angle 2θ (°) of 5.7°±0.5, 7.8°±0.5, 9.1°±0.5, 11.5°±0.5, and 25.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

5. Crystalline Form C of sepiapterin free base of claim 4 having peaks at diffraction angle 2θ (°) of 5.7°±0.5, 7.8°±0.5, 9.1°±0.5, 11.5°±0.5, 15.3°±0.5, 16.0°±0.5, 20.1°±0.5, 25.4°±0.5, and 26.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

6. Crystalline Form C of sepiapterin free base of claim 4 having the X-ray powder diffraction spectrum as shown in FIG. 2.

7. Crystalline Form C of sepiapterin free base of claim 4 having an endotherm at about 58°C, 102°C, 130°C, 156.5°C, or 168°C, in differential scanning calorimetry (DSC) profile.

8. Crystalline Form C of sepiapterin free base of claim 4 having an endotherm at about 58°C, 102°C, 130°C, 156.5°C, and 168°C, in differential scanning calorimetry (DSC) profile.

9. Crystalline Form D of sepiapterin free base having peaks at diffraction angle 2θ (°) of 8.9°±0.5, 10.3°±0.5, 10.9°±0.5, 26.0°±0.5, and 26.8°±0.5, as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

10. Crystalline Form D of sepiapterin free base of claim 9 having peaks at diffraction angle 2θ (°) of 8.9°±0.5, 10.3°±0.5, 10.9°±0.5, 17.8°±0.5, 24.9°±0.5, 26.0°±0.5, 26.7°±0.5, 26.8°±0.5, and 28.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

11. Crystalline Form D of sepiapterin free base of claim 9 having the X-ray powder diffraction spectrum as shown in FIG. 3.

12. Crystalline Form D of sepiapterin free base of claim 9 having an endothermic onset at about 43 °C, 66 °C, or 233°C in differential scanning calorimetry (DSC) profile.

13. Crystalline Form D of sepiapterin free base of claim 9 having an endothermic onset at about 43 °C, 66 °C, and 233°C in differential scanning calorimetry (DSC) profile.

14. Crystalline Form D of sepiapterin free base having peaks at diffraction angle 2θ (°) of about 8.9°, about 10.3°, and about 26.0° as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

15. Crystalline Form D of sepiapterin free base of claim 14 having peaks at diffraction angle 2θ (°) of about 8.9°, about 10.3°, about 10.9°, about 26.0°, and about 26.8° as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

16. Crystalline Form D of sepiapterin free base of claim 15 having peaks at diffraction angle 2θ (°) of about 8.9°, about 10.3°, about 10.9°, about 17.8°, about 24.9°, about 26.0°, about 26.7°, about 26.8°, and about 28.3° as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

17. Crystalline Form F of sepiapterin free base having peaks at diffraction angle 2θ (°) of 9.7°±0.5, 10.2°±0.5, and 11.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

18. Crystalline Form F of sepiapterin free base of claim 17 having peaks at diffraction angle 2θ (°) of 9.7°±0.5, 10.2°±0.5, 11.3°±0.5, 14.0°±0.5, 14.6°±0.5, 19.9°±0.5, 22.2°±0.5, 25.3°±0.5, and 32.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

19. Crystalline Form F of sepiapterin free base of claim 17 having the X-ray powder diffraction spectrum as shown in FIG. 4.

20. Crystalline Form F of sepiapterin free base of claim 17 having an endotherm at about 72°C or 233 °C in differential scanning calorimetry (DSC) profile.

21. Crystalline Form F of sepiapterin free base of claim 17 having an endotherm at about 72 °C and 233 °C in differential scanning calorimetry (DSC) profile.

22. Crystalline Form F of sepiapterin free base having peaks at diffraction angle 2θ (°) of about 9.7°, about 10.2°, and about 11.3° as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

23. Crystalline Form F of sepiapterin free base of claim 22 having peaks at diffraction angle 2θ (°) of about 9.7°, about 10.2°, about 11.3°, about 14.0°, about 14.6°, about 19.9°, about 22.2°, about 25.3°, and about 32.4° as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

24. Crystalline Form G of sepiapterin free base having peaks at diffraction angle 2θ (°) of 10.0°±0.5, 10.6°±0.5, 15.3°±0.5, 24.4°±0.5, 25.7°±0.5, and 26.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

25. Crystalline Form G of sepiapterin free base of claim 24 having peaks at diffraction angle 2θ (°) of 10.0°±0.5, 10.6°±0.5, 11.2°±0.5, 15.3°±0.5, 15.9°±0.5, 22.8°±0.5, 24.4°±0.5, 25.0°±0.5, and 25.7°±0.5, as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

26. Crystalline Form G of sepiapterin free base of claim 24 having the X-ray powder diffraction spectrum as shown in FIG. 5.

27. A pharmaceutical composition comprising the crystalline Form F of sepiapterin free base of claim 17 and a pharmaceutically acceptable carrier.

28. The pharmaceutical composition of claim 27, wherein the crystalline Form F of sepiapterin free base is present in an amount of at least 90 percent by weight of the composition.

29. The pharmaceutical composition of claim 27, wherein the crystalline Form F of sepiapterin free base is formulated as particles less than 100 µm in size.

30. A pharmaceutical composition comprising the crystalline Form F of sepiapterin free base of claim 22 and a pharmaceutically acceptable carrier.

31. A method for preparing a crystalline form of sepiapterin free base comprising preparing a slurry of a first crystalline form of sepiapterin in water, acetone/water, isopropanol/isopropyl acetate, or tetrahydrofuran/n-hexane, isolating crystalline Form D of sepiapterin free base from the slurry, and drying the crystalline Form D.

32. The method of claim 31, further comprising drying crystalline Form D at 40-60 °C for 5-10 hours to produce crystalline Form F of sepiapterin free base.

33. A crystalline form of a salt of sepiapterin, wherein the crystalline form of a salt of sepiapterin is selected from the group consisting of:

(a) a crystalline form of the methanesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.9°±0.5, 23.5°±0.5, and 29.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(b) a crystalline form of the methanesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 21.7°±0.5, 26.1°±0.5, and 28.9°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry
(c) a crystalline form of the nicotinate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 9.5°±0.5, 9.9°±0.5, and 24.5°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(d) a crystalline form of the p-toluenesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.5°±0.5, 15.1°±0.5, and 23.4°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(e) a crystalline form of the benzenesulfonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.5°±0.5, 14.8°±0.5, and 19.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(f) a crystalline form of the phosphate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 16.6°±0.5, 22.2°±0.5, and 25.6°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(g) a crystalline form of the malonate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.9°±0.5, 22.7°±0.5, and 23.8°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(h) a crystalline form of the tartrate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.4°±0.5, 14.2°±0.5, and 21.8°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(i) a crystalline form of the gentisate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.1°±0.5, 8.7°±0.5, and 26.7°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(j) a crystalline form of the fumarate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 11.4°±0.5, 24.0°±0.5, and 28.3°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(k) a crystalline form of the glycolate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.6°±0.5, 10.7°±0.5, and 24.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(l) a crystalline form of the acetate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 6.2°±0.5, 12.0°±0.5, and 18.1°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry;
(m) a crystalline form of the sulfate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 5.1°±0.5, 7.8°±0.5, and 23.0°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry; and

(n) a crystalline form of the sulfate salt of sepiapterin having at least one peak at diffraction angle 2θ (°) of 7.9°±0.5, 8.8°±0.5, and 24.2°±0.5 as measured by X-ray diffractometry by irradiation with Cu Kα X-rays or calculated from X-ray diffractometry.

34. Use of crystalline Form F of sepiapterin free base of claim 17 in the manufacture of a medicament for treating primary tetrahydrobiopterin deficiency in a patient in need thereof.

35. Use of crystalline Form F of sepiapterin free base of claim 17 in the manufacture of a medicament for decreasing phenylalanine levels in a patient in need thereof.

36. Use of crystalline Form F of sepiapterin free base of claim 17 in the manufacture of a medicament for treating phenylketonuria in a patient in need thereof.

37. A method of preparing a liquid formulation comprising dispersing crystalline Form F of sepiapterin free base of claim 17 in a liquid.

38. The method of claim 37, wherein the liquid formulation is a suspension.