fusion protein formulation.

SUMMARY

The present invention aims to solve at least one of the technical problems existing in the prior art to some extent. To this end, the present invention provides a GLP-1-Fc-FGF21 dual-target fusion protein composition, which exhibits strong stability and can extend the shelf life of the product.

In one aspect the present disclosure provides a composition comprising: a GLP-1-Fc-FGF21 fusion protein and a buffer. In the composition of the present invention, the addition of the buffer solution can effectively control the generation of visible particles and aggregates, enhance the stability of the GLP-1-Fc-FGF21 fusion protein, and thereby extend the shelf life of the product.

According to an embodiment of the present invention, the GLP-1-Fc-FGF21 fusion protein comprises amino acid sequences as shown in SEQ ID NO: 1-12.

According to an embodiment of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 1~100 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 2~80 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 2~70 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 5~70 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 2~65 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 2~60 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 2~55 mg/mL.

In some optional embodiments of the present invention, the concentration of the GLP-1-Fc-FGF21 fusion protein in the composition is 10~65 mg/mL.

an injection.

In another aspect the present disclosure provides an injection comprising: (i) a GLP-1-Fc-FGF21 fusion protein, (ii) a histidine buffer, (iii) mannitol, (iv) polysorbate 80 or polysorbate 20; and (v) optionally disodium edetate or calcium disodium edetate, wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1-12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25-100 mg/mL, 25-95 mg/mL, 25-90 mg/mL, 25-85 mg/mL, 25-80 mg/mL, 25-75 mg/mL, 25-70 mg/mL, 25-65 mg/mL, 25-60 mg/mL, 25-55 mg/mL, or 25-50 mg/mL; wherein the concentration of the histidine buffer in the injection is 8-25 mM, preferably 10-20 mM; wherein the concentration of mannitol in the injection is 35-45 mg/mL; wherein the concentration of polysorbate 80 in the injection is 0.4-1.5 mg/mL or 0.4-1.3 mg/mL, or the concentration of polysorbate 20 in the injection is 0.1-0.5 mg/mL; wherein if present the concentration of the metal chelating agent in the injection is 0.05-0.5 mg/mL; wherein the pH of the injection is 6.9-7.5; preferably, wherein the injection does not contain disodium edetate or calcium disodium edetate. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a histidine buffer, mannitol, and optionally disodium edetate or calcium disodium edetate, as well as polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein has an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25~95 mg/mL, 25~90 mg/mL, 25~85 mg/mL, 25~80 mg/mL, 25~75 mg/mL, 25~70 mg/mL, 25~65 mg/mL, 25~60 mg/mL, 25~55 mg/mL, or 25~50 mg/mL; the concentration of the histidine buffer in the composition is 8~25 mM; the concentration of mannitol in the composition is 35~45 mg/mL; the concentration of polysorbate 80 in the composition is 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the composition is 0.1~0.5 mg/mL; the concentration of the metal chelating agent in the composition is 0.05~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain

disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a histidine buffer, mannitol, and optionally disodium edetate or calcium disodium edetate, as well as polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein has an amino acid sequence as shown in SEQ ID NO: 1, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25~50 mg/mL; the concentration of the histidine buffer in the injection is 10~20 mM; the concentration of mannitol in the injection is 35~45 mg/mL; the concentration of polysorbate 80 in the injection is 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the injection is 0.1~0.5 mg/mL; the concentration of the metal chelating agent in the injection is 0.05~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a histidine buffer, sucrose or trehalose, and optionally disodium edetate or calcium disodium edetate, as well as polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25~100 mg/mL; the concentration of the histidine buffer in the injection is 8~25 mM; the concentration of sucrose in the injection is 85~95 mg/mL, or the concentration of trehalose in the injection is 55~65 mg/mL; the concentration of polysorbate 80 in the injection is 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the injection is 0.1~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an

embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a histidine buffer, sucrose or trehalose, and optionally disodium edetate or calcium disodium edetate, as well as polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein has an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25~95 mg/mL, 25~90 mg/mL, 25~85 mg/mL, 25~80 mg/mL, 25~75 mg/mL, 25~70 mg/mL, 25~65 mg/mL, 25~60 mg/mL, 25~55 mg/mL, or 25~50 mg/mL; the concentration of the histidine buffer in the composition is 8~20 mM; the concentration of sucrose in the composition is 85~95 mg/mL; the concentration of trehalose in the injection is 55~65 mg/mL; the concentration of polysorbate 80 in the composition is 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the composition is 0.1~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a histidine buffer, sucrose, and polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in SEQ ID NO: 1, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25~50 mg/mL; the concentration of the histidine buffer in the injection is 10~20 mM; the concentration of sucrose in the injection is 85~95 mg/mL; the concentration of polysorbate 80 in the injection is 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the injection is 0.1~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life. According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate. In another aspect the present disclosure comprising: a GLP-1-Fc-FGF21 fusion protein, a histidine buffer, mannitol, polysorbate 80, and optionally disodium edetate or calcium disodium edetate; wherein the GLP-1-Fc-FGF21

fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1-12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 3-25 mg/mL; wherein the concentration of the histidine buffer in the injection is 5-30 mM, preferably 10-20 mM wherein the concentration of mannitol in the injection is 40-50 mg/mL; wherein the concentration of polysorbate 80 in the injection is 0.1-1.5 mg/mL wherein if present the concentration of the metal chelating agent in the injection is 0.05-0.5 mg/mL; wherein the pH of the injection is 6.4-8.0; preferably, wherein the injection does not contain disodium edetate or calcium disodium edetate. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, sucrose or trehalose, and optionally disodium edetate or calcium disodium edetate, as well as polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 25~100 mg/mL, preferably 25~95 mg/mL, 25~90 mg/mL, 25~85 mg/mL, 25~80 mg/mL, 25~75 mg/mL, 25~70 mg/mL, 25~65 mg/mL, 25~60 mg/mL, 25~55 mg/mL, or 25~50 mg/mL; the concentration of the citrate buffer in the injection is 8~25 mM, preferably 10~20 mM; the concentration of sucrose in the injection is 85~95 mg/mL; the concentration of trehalose in the injection is 55~65 mg/mL; the concentration of polysorbate 80 in the injection is 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the injection is 0.1~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, mannitol, and optionally disodium edetate or calcium disodium edetate, as well as polysorbate 80 or polysorbate 20; wherein the GLP-1-Fc-FGF21 fusion protein comprises an

amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection solution is 25~100 mg/mL, preferably 25~95 mg/mL, 25~90 mg/mL, 25~85 mg/mL, 25~80 mg/mL, 25~75 mg/mL, 25~70 mg/mL, 25~65 mg/mL, 25~60 mg/mL, 25~55 mg/mL, or 25~50 mg/mL; the concentration of the citrate buffer in the injection is 8~25 mM, preferably 10~20 mM; the concentration of mannitol in the injection is 35~45 mg/mL; the concentration of polysorbate 80 in the injection is 0.4~1.5 mg/mL, or 0.4~1.3 mg/mL, or the concentration of polysorbate 20 in the injection is 0.1~0.5 mg/mL; the concentration of the metal chelating agent in the injection is 0.05~0.5 mg/mL; and the pH of the injection is 6.9~7.5. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate. In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, mannitol, polysorbate 80, and optionally disodium edetate or calcium disodium edetate; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 3~25 mg/mL; the concentration of the citrate buffer in the injection is 5~20 mM; the concentration of mannitol in the injection is 40~50 mg/mL; the concentration of polysorbate 80 in the injection is 0.1~1.5 mg/mL; the concentration of the metal chelating agent in the injection is 0.05~0.5 mg/mL; and the pH of the injection is 6.4~8.0. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, mannitol, polysorbate 80, and optionally disodium edetate or calcium disodium edetate; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion

protein in the injection is 3~25 mg/mL; the concentration of the citrate buffer in the injection is 8~20 mM, 10~20 mM, or 10~15 mM; the concentration of mannitol in the injection is 40~50 mg/mL; the concentration of polysorbate 80 in the injection is 0.1~0.5 mg/mL; the concentration of the metal chelating agent in the injection is 0.05~0.5 mg/mL; and the pH of the injection is 6.4~8.0. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, mannitol, polysorbate 80, and optionally disodium edetate or calcium disodium edetate; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in SEQ ID NO: 1, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 3~25 mg/mL; the concentration of the citrate buffer in the injection is 8~20 mM, 10~20 mM, or 10~15 mM; the concentration of mannitol in the injection is 40~50 mg/mL; the concentration of polysorbate 80 in the injection is 0.1~0.5 mg/mL; the concentration of the metal chelating agent in the injection is 0.05~0.5 mg/mL; and the pH of the injection is 6.4~8.0. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, polysorbate 80, and optionally disodium edetate or calcium disodium edetate, as well as sucrose or trehalose; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in any one of SEQ ID NO: 1~12, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 3~25 mg/mL; the concentration of the citrate buffer in the injection is 5~20 mM; the concentration of sucrose in the injection is 80~95 mg/mL; the concentration of trehalose in the injection is 55~65 mg/mL; the concentration of polysorbate

80 in the injection is 0.1~1.5 mg/mL; and the pH of the injection is 6.4~8.0. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In another example of the present invention, it provides an injection. According to an embodiment of the present invention, the injection comprises: a GLP-1-Fc-FGF21 fusion protein, a citrate buffer, polysorbate 80, as well as sucrose or trehalose, and optionally disodium edetate or calcium disodium edetate; wherein the GLP-1-Fc-FGF21 fusion protein comprises an amino acid sequence as shown in SEQ ID NO: 1, and the concentration of the GLP-1-Fc-FGF21 fusion protein in the injection is 3~25 mg/mL; the concentration of the citrate buffer in the injection is 8~20 mM, 10~20 mM, or 10~15 mM; the concentration of sucrose in the injection is 80~95 mg/mL; the concentration of trehalose in the injection is 55~65 mg/mL; the concentration of polysorbate 80 in the injection is 0.1~0.5 mg/mL; and the pH of the injection is 6.4~8.0. The injection of the invention can effectively control the generation of visible particles, improve the physical and chemical stability of the fusion protein, thereby reducing potential safety risks and extending the shelf life.

According to an embodiment of the present invention, the injection does not contain disodium edetate or calcium disodium edetate.

In an optional embodiment of the present invention, the injection described in the aforementioned aspects may further include a preservative.

In an optional embodiment of the present invention, the preservative is at least one selected from phenol, m-cresol, benzyl alcohol, and phenoxyethanol. In an optional embodiment of the present invention, the concentration of phenol in the composition or injection is 0~7 mg/mL, preferably 3~7 mg/mL. In an optional embodiment of the present invention, the concentration of m-cresol in the composition or injection is 0~4 mg/mL, preferably 2~4 mg/mL. In another aspect, the present disclosure provides a use of the composition or injection of the invention for the manufacture of a medicament for treating and/or preventing a metabolic disease; optionally, wherein the metabolic disease is selected from

diabetes, hyperlipidemic obesity, and diseases associated with fatty liver;

optionally, wherein the diseases associated with fatty liver include non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver fibrosis, and cirrhosis.

In another aspect, the present disclosure provides an injection device or container comprising: the aforementioned composition or the aforementioned injection;

optionally, wherein the injection device or container comprises at least one selected from a bottle, a vial, a cartridge, and a syringe;

preferably, wherein the syringe comprises at least one selected from an auto-injector and a pre-filled syringe.

Additional aspects and advantages of the invention will be partially set forth in the following description, and will partially become apparent from the following description, or may be learned through the practice of the invention.

Description of the drawings

The above and/or additional aspects and advantages of the present invention will become apparent and readily understood from the description of the embodiments in conjunction with the accompanying drawings, wherein:

Figure 1 shows the high-temperature SEC (Size Exclusion Chromatography) aggregate growth trend chart of different formulations in step 7 of Example 1 of the invention;

Figure 2 shows the high-temperature RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) purity (total related substances) growth trend chart of different formulations in step 7 of Example 1 of the invention.

EXAMPLES

The embodiments of the present invention are described in detail below. The embodiments described below are exemplary and are intended solely to explain the invention, and should not be construed as limiting the invention.

It should be noted that the terms "first" and "second" are used for descriptive purposes only