NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 1903.52-2021

National food safety standard - Food Nutrient Fortifier - Heme Chloride

Issued on: September 7, 2021 **Implemented on: March 7, 2022**

Issued by: National Health Commission;
State Administration for Market Regulation

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1 Scope

This Standard applies to the food nutrient fortifier heme chloride that is made by using glacial acetic acid method, enzymatic hydrolysis and acetic acid combination method, with the blood and blood cell powder of qualified quarantine animals as the main raw materials.

2 Molecular formula, structural formula and relative

molecular mass

2.1 Molecular formula

C34H32ClFeN4O4

2.3 Relative molecular mass

651.95 (according to the international relative atomic mass in 2018)

3.1 Sensory requirements

Sensory requirements shall be in accordance with Table 1.

Table 1 - Sensory requirements

Item Requirement Inspection method Color Blue or black

Appendix A Inspection methods

A.1 General provisions Unless otherwise stated, only use confirmed analytical reagents and grade-3 water that is specified in GB/T 6682 during the analysis. The standard titration solutions, standard solutions for impurity determination, preparations and products, which are used in the test method, shall be prepared in accordance with the provisions of GB/T 601, GB/T 602, and GB/T 603, when other requirements are not specified. The used

solution, if not indicated which solvent is used, refers to aqueous solution.

A.2 Identification test A.2.1 Reagents and materials A.2.1.1 Nitric acid: analytically pure.

A.2.1.2 Sodium sulfide: analytically pure.

A.2.1.3 Sulfuric acid solution: Take 5 mL of concentrated sulfuric acid; slowly add into about 80 mL of water; then, add water to make the volume up to 100 mL.

A.2.1.4 Hydrochloric acid solution: Take 50 mL of concentrated hydrochloric acid; add water to dilute to make the volume 100 mL.

A.2.1.5 Ammonium thiocyanate solution: Dissolve 8 g of ammonium thiocyanate in an appropriate amount of water; use water to dilute to 100 mL.

A.2.1.6 Pyridine sodium hydroxide solution: Dissolve 1.2 g of sodium hydroxide in 200 mL of water; then, add 100 mL of pyridine and mix.

A.2.1.7 Ammonia solution: Take 400 mL of ammonia water; use water to dilute to 1 000 mL.

A.2.2 Instruments and apparatuses A.2.2.1 Water bath tank.

A.2.2.2 Analytical balance: Sensitivity is 0.1 g and 0.001 g.

A.2.3 Identification method A.2.3.1 Color rendering test A.2.3.1.1 Weigh 0.01 g (accurate to 0.001 g) of heme chloride sample; add 1 mL of sulfuric acid solution (A.2.1.3) and 1 mL of nitric acid (A.2.1.1) to dissolve;

evaporate to dryness in a water bath. Add 10 mL of hydrochloric acid solution (A.2.1.4) to the residue to dissolve; add a few drops of ammonium thiocyanate solution (A.2.1.5); shake well; the solution shall appear dark red.

A.2.3.1.2 Weigh 0.005 g (accurate to 0.001 g) of heme chloride sample; add 10 mL of pyridine sodium hydroxide solution (A.2.1.6) to dissolve; then, add 0.1 g of sodium sulfide; shake well. The solution shall appear dark red.

A.2.3.1.3 Weigh 0.01 g (accurate to 0.001 g) of heme chloride sample; add 5 mL of nitric acid; shake well; heat. The solution shall appear yellow. After cooling, use ammonia solution (A.2.1.7) to adjust the pH to alkaline; the solution shall appear orange-yellow.

A.2.3.2 Infrared spectroscopy Use potassium bromide pellet technique to test in accordance with the provisions of GB/T 6040; the infrared spectrum of the sample shall be consistent with the control spectrum. See Appendix B for the control spectrum.

A.3 Determination of heme chloride ($C_{34}H_{32}ClFeN_4O_4$) content A.3.1 Ultraviolet spectrophotometry A.3.1.1 Method summary After the heme chloride is dissolved in 0.1 mol/L sodium hydroxide solution, use the ultraviolet spectrophotometry for determination. There is a maximum absorption peak at 386 nm +/- 1 nm. Measure the absorbance value A; calculate the sample content according to the concentration of the standard working

solution.

A.3.1.2 Reagents and materials A.3.1.2.1 0.1 mol/L sodium hydroxide solution: Weigh 4.0 g of sodium hydroxide;

add an appropriate amount of water to dissolve; use water to dilute to 1 L.

A.3.1.2.2 Heme chloride standard substance (Hemin): C34H32ClFeN4O4, CAS:

16009-13-5, purity >=97%.

A.3.1.2.3 Heme chloride standard stock solution: accurately weigh 20 mg (accurate to 0.1 mg) of heme chloride standard substance; add 0.1 mol/L sodium hydroxide solution (A.3.1.2.1) to a constant volume of 100 mL; prepare a standard stock solution of a concentration of 200.0 mg/L. Store the solution m - the mass of the sample represented by the final sample solution, in grams (g).

The test results are based on the arithmetic mean of the parallel determination results, and the absolute difference between two independent determination results obtained under repeatability conditions is not more than 5% of the arithmetic mean.

A.3.2 High-performance liquid chromatography A.3.2.1 Method summary After the heme chloride sample is dissolved and extracted with 0.1 mol/L sodium hydroxide solution, use high performance liquid chromatography to measure. The retention time is qualitative, and the peak area is quantified by external standard method.

A.3.2.2 Reagents and materials A.3.2.2.1 Methanol: chromatographic pure.

A.3.2.2.2 Acetic acid: chromatographic pure.

A.3.2.2.3 Acetic acid solution: draw 6 mL of acetic acid (A.3.2.2.2); use water to dilute to 1 L.

A.3.2.2.4 Heme chloride standard substance (Hemin): See A.3.1.2.2.

A.3.2.2.5 Heme chloride standard stock solution: See A.3.1.2.3.

A.3.2.2.6 Heme chloride standard working solution: Use 0.1 mol/L sodium hydroxide solution to dilute the heme chloride standard stock solution to prepare the corresponding standard working solution before use.

A.3.2.2.7 Filter membrane: 0.45 um, water phase.

A.3.2.3 Instruments and apparatuses A.3.2.3.1 High performance liquid chromatograph: equipped with ultraviolet detector or diode array detector.

A.3.2.3.2 Analytical balance: sensitivity of 0.1 g and 0.000 1 g.

A.3.2.4 Analysis steps Weigh 0.02 g (accurate to 0.001 g) of sample in a 100 mL volumetric flask; use

0.1 mol/L sodium hydroxide solution to dissolve it and dilute it to the mark; shake well; then, use it for HPLC determination.