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Foreign Agricultural Service

GAIN Global Agricultural Information Network

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Report Name: National Food Safety Standard of Food Nutrition Fortifier Heme Iron Notified to WTO

Country: China - People's Republic of

Post: Beijing

Report Category: FAIRS Subject Report, Sanitary/Phytosanitary/Food Safety, WTO Notifications

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Report Highlights:

On March 6, 2023, China notified a new National Food Safety Standard of Food Nutrition Fortifier Heme Iron to the World Trade Organization (WTO) under G/SPS/N/CHN/1267. The deadline for comment submission is May 5, 2023. The proposed date of entry into force is to be determined. Comments may be submitted by email to China's SPS Enquiry Point at sps@customs.gov.cn. This report provides an unofficial translation of the draft standard.

Summary:
On March 6, 2023, China notified a new National Food Safety Standard of Food Nutrition Fortifier Heme Iron to the WTO under G/SPS/N/CHN/1267. This standard applies to the food nutrient fortifier heme iron prepared by enzymolysis, separation, and drying with animal blood or blood cell liquid obtained by centrifugation as raw materials. This standard mainly specifies the technical requirements and testing methods of food nutritional fortifier heme iron. This report provides an unofficial translation of the draft standard. Comments on this Standard may be submitted by email to China's SPS Enquiry Point at sps@customs.gov.cn.

BEGIN TRANSLATION

National Food Safety Standard
Food Nutrition Fortifier Heme Iron
(Draft for comments)

1 Scope

This standard is applicable to heme iron, a food nutrition fortifier, which is made through enzymolysis, separation and drying from animal blood that meet inspection and quarantine requirements or blood cell liquid obtained by centrifugation as raw materials.

2 Chemical name, molecular formula, structural formula, and relative molecular mass

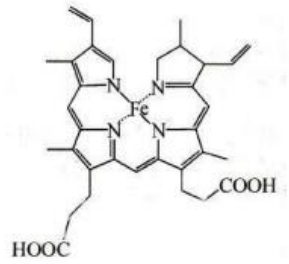
2.1 Chemical name

Heme iron

2.2 Molecular formula

$C_{34}H_{32}FeN_4O_4$

2.3 Structural formula



2.4 Relative molecular mass

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

3 Technical requirements

3.1 Sensory requirements

Sensory requirements shall comply with the provisions in Table 1.

Table 1: Sensory requirements

Item	Requirements	Testing Methods
Color	Reddish brown to dark brown	Take an appropriate amount of sample and put it in clean and dry transparent glassware. Observe the color and state under natural light, smell the odor, and observe the impurities.
State	Powder or granule	
Odor	Tasteless or slight odor of heme iron.	
Impurities	There is no visible impurity.	

3.2 Physical and chemical indicators

Physical and chemical indicators shall conform to the provisions of Table 2.

Table 2: Physical and chemical indicators

Item		Indicators	Testing Methods
Iron (Fe), w/%		1.0-2.6	GB 5009.90
Heme iron ($C_{34}H_{32}FeN_4O_4$), *w*/%		9.0-27.0	A.3 in Appendix A
Drying loss, w/%	≤	6.0	GB5009.3 (Direct drying method)
Burned residues, w/%	≤	12.0	GB 5009.4
Lead (Pb) / (mg/kg)	≤	2.0	GB 5009.75 or GB 5009.12
Total arsenic (As)/ (mg/kg)	≤	2.0	GB 5009.76 or GB 5009.11

3.3 Microbial limits

The microbial limits shall comply with the provisions in Table 3.

Table 3: Microbial limits

Item	Limit [a]				Testing methods
	n	c	m	M	
Salmonella/25g	5	0	0	-	GB 4789.4
Staphylococcus aureus/(CFU/g)	5	1	100	1,000	GB 4789.10 Method II
[a] Sampling and processing of the samples shall be carried out according to GB 4789.1.					

Appendix A
Testing Methods

A.1 General Provisions

The reagents and water used in the testing methods of this standard refer to analytical pure reagents and Level III water specified in GB/T 6682 unless otherwise specified. The solution used in the tests refers to aqueous solution unless otherwise specified, and all solutions expressed by "%" are mass fractions.

A.2 Identification Test

A.2.1 Reagents and materials

A.2.1.1 Sodium hydroxide

A.2.1.2 Pyridine

A.2.1.3 Sodium thiosulfate

A.2.1.4 Nitric acid: chromatographically pure

A.2.1.5 Ammonia water

A.2.1.6 1 mol/L sodium hydroxide solution: weigh 40.0 g sodium hydroxide and dissolve in water without carbon dioxide and volume to the scale of 1 L.

A.2.1.7 Pyridine sodium hydroxide solution: take 100 mL of Pyridine, add 30 mL of 1 mol/L sodium hydroxide solution and dilute it to 300 mL with water.

A.2.2 Instruments and equipment

A.2. 2.1 Analytical balance: sensitivity 0.0001g

A.2. 2.2 Water bath tank

A.2.3 Identification method

A.2.3. 1 Coloring test
Dissolve 10 mg of heme iron in 50 mL of pyridine sodium hydroxide solution, take 5 mL of the solution and add 15 mg of sodium thiosulfate. The solution turns red.

A.2.3.2 Coloring test
Put 10 mg of heme iron in a 100 mL beaker, add 5 mL of nitric acid, heat it until it turns yellow. Cool it and alkalize it with ammonia water until it turns orange.

A.2.3.3 Ultraviolet spectrometry
Dissolve 10 mg of heme iron in 50 mL of pyridine sodium hydroxide solution. It reaches the

maximum absorption at the wavelength of 399 nm in the ultraviolet scanning spectrum.

A.3 Determination of heme iron ($C_{34}H_{32}FeN_4O_4$)

A.3.1 Summary of the method

Dissolve heme iron in the sample with pyridine sodium hydroxide, separate with C_{18} chromatographic column then determine with liquid chromatography.

A.3.2 Reagents and materials

Unless otherwise specified, all reagents used are analytically pure, and the water is Level I water specified in GB/T 6682.

A.3.2.1 Methanol: chromatographically pure.

A.3.2.2 Formic acid: chromatographically pure.

A.3.2.3 Sodium hydroxide

A.3.2.4 Pyridine

A.3.2.5 Hemoglobin ($C_{34}H_{33}FeN_4O_5$, CAS No.: 15489-90-4) standard substance: content is not less than 298.0%.

A.3.2.6 1 mol/L sodium hydroxide solution: weigh 40.0g sodium hydroxide, dissolve in water without carbon dioxide and volume to 1 L.

A.3.2.7 Pyridine sodium hydroxide solution: take 100 mL of Pyridine, add 30 mL of 1 mol/L sodium hydroxide solution and dilute with water to 300 mL.

A.3.2.8 Standard stock solution of hemoglobin: accurately weigh 0.1028g of hemoglobin, dry for 3 hours at 100℃, dissolve it with this pyridine sodium hydroxide solution (A.3.2.7), and volume to the scale of 100 mL, which is equivalent to 1 mg/mL heme iron. Store at 0-4℃ in the dark and shall not be kept for more than a week.

A.3.3 Instruments and equipment

A.3.3.1 High performance liquid chromatograph: equipped with ultraviolet detector or diode array detector.

A.3.3.2 Analytical balance: sensitivity of 0.0001g

A.3.3.3 Ultrasonic generator: the power is greater than 180 W.

A.3.3.4 Centrifuge: greater than 4,000 r/minute.

A.3.3.5 Liquid chromatography reference conditions

A.3.3.5.1 Chromatographic column: C_{18} column with a length of 250 mm, an inner diameter of 4.6 mm and a particle size of 5 gm or equivalent chromatographic column.

A.3.3.5.2 Mobile phase: methanol +0.1% formic acid aqueous solution (70+30, V+V).

A.3.3.5.3 Flow rate: 1.0 mL/min.

A.3.3.5.4 Column temperature: 30℃.

A.3.3.5.5 Sample volume: 5.0 μL.

A.3.3.5.6 Detection wavelength: 399 nm.

A.3.3.6 Analysis steps
Accurately weigh 0.1g of the sample (accurate to 0.001g), dissolve it with pyridine sodium hydroxide solution (A.3.2.7), and assist the solution with ultrasonic wave for 10 minutes in the dark. Then transfer it to a 50 mL volumetric flask, volume to the scale with pyridine sodium hydroxide solution and mix well. Take 1 mL of this solution, dilute it to 10 mL with pyridine sodium hydroxide solution and centrifuge it at 4,000 r/min for 10 minutes. The supernatant is filtered through 0.45 μm membrane and determined by liquid chromatography. At the same time, conduct a blank test and follow the same operations as the determination of samples except that no sample is added.

A.3.4 Qualitative analysis
According to the method of qualitative identification based on retention time consistency, the chromatographic peak of heme iron in the sample is determined according to the retention time of standard heme iron substance (see Appendix B).

A.3.5 Quantitative analysis
Put 0.25 ml, 0.50 mL, 1.00 mL, 2.00 mL, 3.00 mL and 5.00 mL, of the standard stock solution of hemoglobin (A.3.2.8) respectively into 50 mL volumetric flasks, volume with pyridine sodium hydroxide solution (A.3.2.7). Prepare a standard solution that is equivalent to the heme iron solution with a concentration of 5 μg/mL, 10 μg/mL, 20 μg/mL, 40 μg/mL, 60 μg/mL, and 100 μg/mL, respectively, and determine by liquid chromatography. Draw standard curves with heme iron concentration as abscissa and chromatographic peak area as ordinate.

The standard working curve is used to quantify the sample, and the response values of heme iron in the standard solution and the sample shall be within the detection linear range of the instrument.

Under the chromatographic conditions in A.3.3.5, see Appendix B for the chromatograms of the standard hemoglobin substance and heme sample.

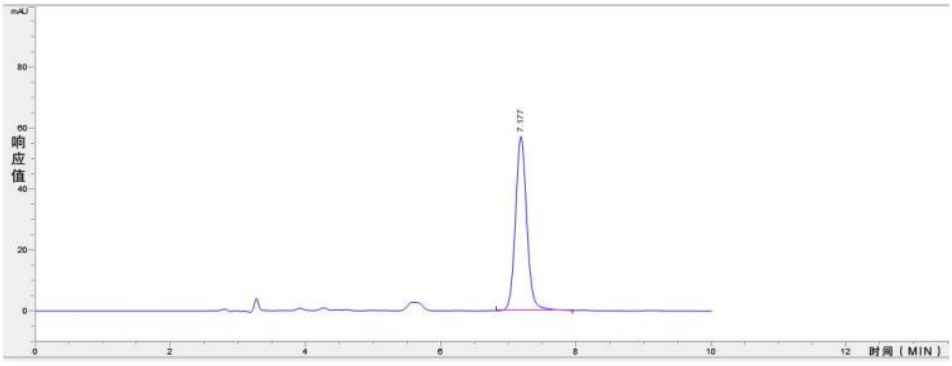
A.3.6 Calculation
The mass fraction w (%) of heme iron ($C_{34}H_{32}FeN_4O_4$) is calculated according to formula (A.1):

$$w_1(\%) = \frac{(c-c_0) \times V \times f}{m \times 10^6} \times 100\% \cdot \quad \text{(A.1)}$$

Where:
c - Mass concentration of heme iron in the sample solution, in microgram per milliliter (μg/ml),
c_0 - Mass concentration of heme iron in blank test, in microgram per milliliter (μg/mL),
V - Total volume of the sample at constant volume, in milliliter (mL),
f - Sample dilution factors,
m - Weighed sample mass, in grams (g),
The arithmetic average of the parallel determination results is taken as the determination result and the absolute difference between the two parallel determination results is not more than 2.0%.

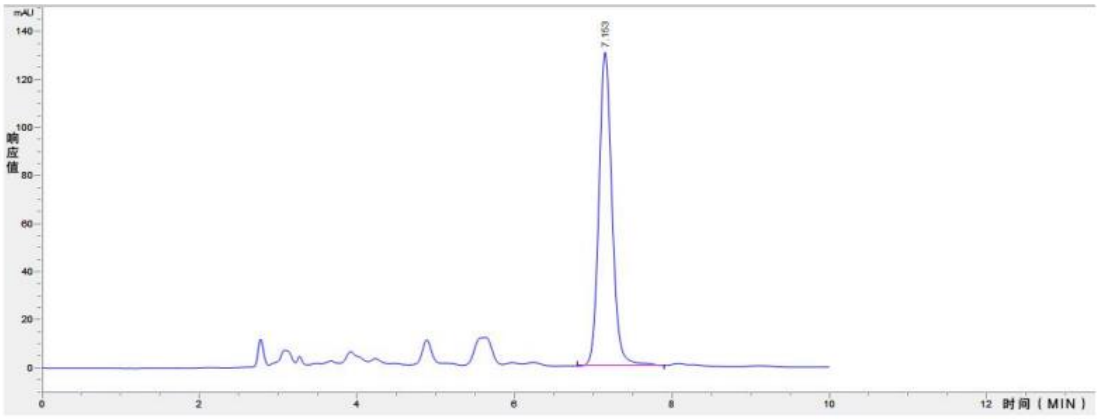
Appendix B
Liquid Chromatogram

The liquid chromatogram of the standard hemoglobin substance is shown in Figure B.1.



B.1 Liquid Chromatogram of Standard Hemoglobin Substance (the concentration is calculated by heme iron)

The liquid chromatogram of heme iron sample is shown in Figure B.2.



B.2 Liquid Chromatogram of Heme Iron Sample

END TRANSLATION

Attachments:

No Attachments.