FERROUS GLYCINATE (processed with CITRIC ACID)

New specifications prepared at the 61st JECFA (2003), published in FNP 52 Add 11 (2003). The compound is suitable for use as a source of iron, provided that the total intake of iron does not exceed the provisional maximum tolerable daily intake of 0.8 mg/kg of body weight for iron from all sources allocated by the 27th meeting.

SYNONYMS Ferrous bisglycinate

DEFINITION Ferrous glycinate is an iron (II) chelate with an iron to glycine ratio of 1:2. Ferrous glycinate (processed with citric acid) also contains citric acid. It is manufactured by reaction of reduced iron with glycine in the presence of citric acid. The resulting product is spray dried without prior removal of the citric acid. The substance is highly hygroscopic and may contain water in variable amounts.

Assay Not less than 21.6% and not more than 23.0% of iron (II) on the dried basis

DESCRIPTION A free-flowing, grey green, hygroscopic, fine powder.

FUNCTIONAL USES Nutrient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water, practically insoluble in acetone and in ethanol

Ferrous ion (vol. 4) Passes test

Infrared spectrum (Vol. 4) The infrared spectrum of a potassium bromide dispersion of the sample dried at 105° overnight corresponds to the reference infrared spectrum below.

PURITY

Loss on drying (Vol. 4) Not more than 9% (105°, 3 h)

Citric acid Not more than 19.5% on the dried basis
See description under TESTS

Total iron Between 19 and 24% on the dried basis
See description under TESTS

Ferric iron Not more than 4% on the dried basis
See description under TESTS

Nitrogen (Vol. 4) Between 11 and 12% on the dried basis

Lead (Vol. 4) Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described on Volume 4, "Instrumental

methods".

TESTS

PURITY TESTS

Citric acid

Determine by HPLC using the following conditions:

Column:	C18 reversed phase (4.6 x 100 mm)
Detection:	UV at 206 nm.
Mobile phase:	acetonitrile: 0.1 M diammonium hydrogen phosphate (3:97).
Flow rate:	1.0 ml/min.
Injection:	10 μl.
Run time:	10 min.
Retention time for citric acid:	about 3 min.

0.1 M diammonium hydrogen phosphate: Aqueous solution of 13.20 g/l of diammonium hydrogenphosphate, $(NH_4)_2HPO_4$, pH adjusted to 2.5 with phosphoric acid TS.

Standard stock solution: Accurately weigh about 200 mg of anhydrous citric acid, dissolve in 0.1 M diammonium hydrogen phosphate and dilute to 100.0 ml.

Calibration solution: Dilute 10.00 ml of the standard stock solution to 50.0 ml with 0.1 M diammonium hydrogenphosphate.

Sample solution: Accurately weigh about 200 mg of the dried sample, dissolve in 0.1 M diammonium hydrogen phosphate and dilute to 100.0 ml.

Procedure
Separately inject equal amounts of the sample solution and the calibration solution into the chromatograph. Record the chromatograms and measure the response of citric acid peak. Calculate the content of citric acid in the sample by the following formula:

$$\% \text{ citric acid on the dried basis} = \frac{A_S \times W_C \times 50 \times 100}{A_C \times W_S \times 10}$$

where,
A_S = Peak area of sample solution
A_C = Peak area of calibration solution
W_S = Weight of dry sample in g
W_C = Weight of anhydrous citric acid in g

Total iron

Accurately weigh about 0.500 g of thoroughly homogenised sample, and place the sample into a digestion vessel. Add 5 ml of concentrated nitric acid, mix the slurry, and cover the vessel with a watch glass or vapour recovery device. Without bringing to a boil, heat the sample at 95±5° for a period of 30 to 40 min. If brown fumes evolve following heating for the prescribed time, add 2 ml of concentrated nitric acid repeatedly, with heating for 15 to 20 min, until brown fumes cease to evolve. Continue heating the sample digest until the volume has been reduced to about 3 ml, ensuring that the bottom of the vessel is covered with the sample digest at all times. Remove the heating vessel from the heating source, and allow its contents to cool thoroughly. Add 2 ml of concentrated hydrochloric acid to the sample digest,

and cover with a watch glass. Replace the vessel on the heating source, and reflux the sample digest at 95±5° for 15 to 20 min. Before removing the vessel from the heating source, verify that the evolving vapour is clear. Add 2 ml of water and bring the solution to boiling. Allow to cool to room temperature, dilute to 50 ml with water, add 3 g of potassium iodide, shake well, and let stand in the dark for 5 min. Titrate any liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator; 1 ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of total iron.

Ferric iron

Accurately weigh about 5 g of the sample into a 250 ml glass-stoppered flask, and dissolve in a mixture of 100 ml of water and 10 ml of hydrochloric acid. To the solution, add 3 g of potassium iodide, shake well, and let stand in the dark for 5 min. Titrate any liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator; 1 ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of ferric iron.

METHOD OF ASSAY

Dissolve about 1 g, accurately weighed, of the dried sample in a mixture of 150 ml of water and 10 ml of sulfuric acid TS in a 300-ml flask. Add 1 drop of orthophenanthroline TS, and immediately titrate with 0.1 *N* ceric sulfate. Perform a blank determination in an identical manner, and perform any necessary correction; 1 ml of 0.1 *N* ceric sulfate is equivalent to 5.585 mg of ferrous ion.

FTIR spectrum

The absorptions around 2250 cm^{-1} are due to CO_2 absorption