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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.249-2016

**National food safety standard -
Determination of ferrous sodium
ethylenediaminetetraacetate in iron fortified soy sauce**

食品安全国家标准

铁强化酱油中乙二胺四乙酸铁钠的测定

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National food safety standard -

Determination of ferrous sodium

ethylenediaminetetraacetate in iron fortified soy sauce

1 Scope

This standard specifies the method for the determination of ferrous sodium ethylenediaminetetraacetate in iron fortified soy sauce.

This standard applies to the determination of ferrous sodium ethylenediaminetetraacetate in iron fortified soy sauce.

2 Principles

The specimen is precipitated by methanol and filtered, the filtrate is diluted and separated by reversed-phase high-performance liquid chromatography. It is detected by the ultraviolet detector at a wavelength of 254 nm, the retention time of the chromatographic peak is used for qualitative determination, the external standard method is used for quantification.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade, the water is the grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH_3OH).

3.1.2 Methanol (CH_3OH): chromatographically pure.

3.1.3 Water solution of tetrabutylammonium hydroxide ($\text{C}_{16}\text{H}_{36}\text{N} \cdot \text{OH}$) (40 g/100 mL).

3.1.4 Formic acid (HCOOH).

3.1.5 Formic acid solution (100 mL/L): PIPETTE 5 mL of formic acid; USE water to make its volume reach to 50 mL.

3.1.6 Aqueous methanol solution (3 + 1): TAKE 750 mL of methanol (3.1.1);

ADD 250 mL of water; MIX it uniformly.

3.1.7 Ferrous sodium ethylenediaminetetraacetate standard substance ($\text{C}_{10}\text{H}_{12}\text{FeN}_2\text{NaO}_8 \cdot 3\text{H}_2\text{O}$): purity $\geq 99.0\%$.

3.2 Preparation of standard solution

3.2.1 Ferrous sodium ethylenediaminetetraacetate standard stock solution (2.0 mg/mL): WEIGH 0.05 g of ferrous sodium ethylenediaminetetraacetate standard substance (accurate to 0.0001 g); PLACE it in a 25 mL volumetric flask; USE water to dissolve it to make it reach to a certain volume. This solution shall be prepared in a brown volumetric flask, stored in a refrigerator in the dark, recommended for use within 15 days.

3.2.2 Ferrous sodium ethylenediaminetetraacetate standard intermediate solution (100 $\mu\text{g/mL}$): USE the aqueous methanol solution (3.1.6) to make the volume of 2.5 mL of ferrous sodium ethylenediaminetetraacetate standard stock solution to 50 mL. This solution shall be prepared in a brown volumetric flask and stored in the refrigerator in the dark.

3.2.3 Ferrous sodium ethylenediaminetetraacetate standard working solution: Respectively, PIPETTE 1.0 mL, 2.0 mL, 4.0 mL, 6.0 mL, 8.0 mL, 10.0 mL of ferrous sodium ethylenediaminetetraacetate standard intermediate solution in a 50.0 mL brown volumetric flask; USE water to make its volume reach to the mark. The concentration is 2.0 $\mu\text{g/mL}$, 4.0 $\mu\text{g/mL}$, 8.0 $\mu\text{g/mL}$, 12.0 $\mu\text{g/mL}$, 16.0 $\mu\text{g/mL}$, 20.0 $\mu\text{g/mL}$, which shall be prepared before use.

4 Instruments and equipment

4.1 High-performance liquid chromatograph, which is equipped with UV detector or diode array detector.

4.2 Balance: The sensitivity is 0.1 mg.

4.3 pH meter: Accuracy is 0.01.

5 Analytical procedures

5.1 Preparation of sample solution

PIPETTE 2.50 mL of the uniformly-mixed specimen; PLACE it into a 50.0 mL brown volumetric flask; ADD aqueous methanol solution (3.1.6) to dilute it to the mark; SHAKE it uniformly. LET it be standing in the dark for 50 min; USE filter paper to filter it. PIPETTE 5.00 mL of filtrate; PLACE it into a 50 mL brown volumetric flask; USE water to dilute it to the mark; SHAKE it uniformly; USE 0.45 μm filter membrane to filter it, to obtain a sample solution, which is ready

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