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National food safety standard – Determination of ethylenediaminetetraacetates in foods

食品安全国家标准

食品中乙二胺四乙酸盐的测定

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1 Scope

This Standard specifies the liquid chromatography for the determination of ethylenediaminetetraacetates in foods.

This Standard applies to the determination of disodium ethylenediaminetetraacetate dihydrate in jams, preserved fruits, canned pickled vegetables, vegetable purees (sauces) except tomato sauce, canned nuts and seeds, canned mixed congees, compound seasonings and beverages, and the determination of calcium disodium ethylenediaminetetraacetate hydrate in compound seasonings. This Standard does not apply to the determination of sodium iron (III) ethylenediaminetetraacetate trihydrate in iron fortified soy sauce.

2 Principle

Sample of disodium ethylenediaminetetraacetate dihydrate to be tested is extracted using water; the extract is added with ferric chloride for complexation; it is then purified using the ProElut PXA solid-phase extraction column, tested using the liquid chromatography and quantitated using the external standard method. During the determination of compound seasonings: first determine the total content of ferric chloride complex of ethylenediaminetetraacetates; determine the content of calcium disodium ethylenediaminetetraacetate hydrate; then determine the content of disodium ethylenediaminetetraacetate dihydrate based on the two results.

3 Reagents and materials

Unless specified otherwise, all reagents used in this Method are analytically pure; the water is grade one water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH_3OH): chromatographically pure.

3.1.2 Hydrochloric acid (HCl).

working solutions of 0.5 μg/mL, 2.0 μg/mL, 5.0 μg/mL, 10.0 μg/mL and 50.0 μg/mL.

3.4.3 Calcium disodium ethylenediaminetetraacetate hydrate standard solution: accurately weigh an appropriate amount of calcium disodium ethylenediaminetetraacetate hydrate standard substance; use water to dissolve and make up to a brown volumetric flask of 100 mL to obtain a standard stock solution of concentration 10 mg/mL; transfer the solution to a receiver for storage below 4°C.

3.4.4 Calcium disodium ethylenediaminetetraacetate hydrate standard serial solutions: absorb 1 volume of calcium disodium ethylenediaminetetraacetate hydrate standard solution and 9 volumes of water to dilute to a standard medium solution of concentration 1 mg/mL. Then use water to dilute the calcium disodium ethylenediaminetetraacetate hydrate standard solution to serial standard working solutions of 1.0 μg/mL, 10.0 μg/mL, 20.0 μg/mL, 50.0 μg/mL and 100.0 μg/mL.

4 Apparatus

4.1 Liquid chromatograph: equipped with an ultraviolet detector.

4.2 Electronic balance: sensitivities 0.1 mg and 0.01 g.

4.3 Centrifugal machine: rotational speed ≥ 7,500 r/min.

4.4 Vortex oscillator.

4.5 Ultrasonic cleaner.

4.6 pH meter: accuracy 0.01.

4.7 24-hole solid phase extraction apparatus.

4.8 Micropore filter membrane: 0.45 μm.

4.9 ProElut PXA solid phase extraction column: 150 mg/6 mL or equivalent. The main ingredient of PXA is quaternary ammonium groups bonded on the copolymer of polystyrene and divinyl benzene containing hydrophilic groups.

5 Analytical procedures

5.1 Sample preparation

5.1.1 Solid sample

Use a pulverizer to crush sample; mix up before loading into a clean container; seal and mark the container. Sample having a high content of sugar needs to be frozen

(only referring to compound seasonings)

5.2.2.1 Extraction

As in 5.2.1.1.

5.2.2.2 Purification

Use in succession 5 mL of methanol and 5 mL of water to activate the PXA column; pass all solution to be purified through the column; use in succession 5 mL of water and 5 mL of methanol to rinse; drain; use 5 mL of 5% formic acid-methanol aqueous solution for elution; drain; collect the eluant to make up to 5 mL; filter; determine on a liquid chromatograph.

5.3 Instrument reference conditions

a) chromatographic column: Plus-C_{18} column, 250 mm in length, 4.6 mm in inner diameter and 5.0 μm in grain size, or an equivalent chromatographic column;

b) moving phase for the detection of disodium ethylenediaminetetraacetate dihydrate complex: methanol: tetrabutylammonium bromide-sodium acetate mixed solution (pH 4.0) = 15:85;

c) moving phase for the detection of calcium disodium ethylenediaminetetraacetate hydrate: tetrabutylammonium bromide-sodium acetate mixed solution (pH 2.5) = 15:85;

d) flow rate: 0.8 mL/min;

e) column temperature: 35°C;

f) wavelength: 254 nm;

g) volume of sample: 10 μL.

5.4 Plotting of standard curve

5.4.1 Determination of total content of ethylenediaminetetraacetates

Absorb respectively 0 μL, 25 μL, 100 μL, 250 μL, 500 μL and 2,500 μL of disodium ethylenediaminetetraacetate dihydrate standard medium solution of concentration 1 mg/mL to pour into a colourimetric tube of 50 mL; add 25 mL of water and 1.0 mL of ferric chloride solution; use vortex to mix up; carry out ultrasonic extraction for 20 min; cool to room temperature before using water to make up to scale; the concentrations of the standard serial working solutions obtained through complexation are respectively 0 μg/mL, 0.5 μg/mL, 2.0 μg/mL, 5.0 μg/mL, 10.0 μg/mL and 50.0 μg/mL. Then inject them into the liquid chromatograph; measure the corresponding peak

m_1 – the mass of sample weighed, in g;

1,000 – the conversion coefficient.

The result of calculation is deducted by blank value. The result of calculation is rounded off to three significant digits.

6.2 Compound seasonings

The content of ethylenediaminetetraacetates in sample shall be expressed by dividing into disodium ethylenediaminetetraacetate dihydrate and calcium disodium ethylenediaminetetraacetate hydrate. The content of calcium disodium ethylenediaminetetraacetate hydrate is calculated in accordance with Equation (2):

$$X_2 = \frac{\rho_2 \times V}{m_2} \times \frac{1\,000}{1\,000} \quad \cdots\cdots (2)$$

where:

X_2 – the content of calcium disodium ethylenediaminetetraacetate hydrate in sample, in mg/kg;

ρ_2 – the mass concentration of calcium disodium ethylenediaminetetraacetate hydrate in sample solution, μg/mL/mL;

V – the total volume of sample to be tested, in mL;

m_2 – the mass of sample weighed, in g;

1,000 – the conversion coefficient.

The result of calculation is deducted by blank value. The result of calculation is rounded off to three significant digits.

The content of disodium ethylenediaminetetraacetate dihydrate is calculated in accordance with Equation (3):

$$X_3 = \left(X_1 \times \frac{292.24}{336.21} - X_2 \times \frac{292.24}{376.21}\right) \times \frac{336.21}{292.24} \quad \cdots\cdots (3)$$

where:

X_3 – the content of disodium ethylenediaminetetraacetate dihydrate in sample, in mg/kg;

X_1 – the content of disodium ethylenediaminetetraacetate dihydrate obtained from the calculation of disodium ethylenediaminetetraacetate dihydrate complex in sample, in mg/kg;

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