NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.129-2023

National food safety standard - Determination of ethoxyquin in fruits

食品安全国家标准 食品中乙氧基喹的测定

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Standardization Administration of the PRC

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Foreword

This document was issued on 6 September 2023 by the State Administration for Market Regulation; Standardization Administration of the PRC and takes effect on 6 March 2024.

It is a GB standard without the /T suffix: compliance is mandatory in China.

1 Scope

GB 5009.129-2023 covers the determination of ethoxyquin in food, applied to fresh fruit, by two methods set out side by side in the same document. In the first, the residue is extracted from the sample with n-hexane under alkaline conditions, blown to dryness under nitrogen, taken up in acetonitrile containing ascorbic acid and measured by liquid chromatography with fluorescence detection against external standards; the detection limit is 0.01 mg/kg and the quantitation limit 0.02 mg/kg. The second extracts in the same way but redissolves in n-hexane and measures by gas chromatography with a nitrogen and phosphorus detector, which gives a laboratory without a fluorescence detector a route to the same

answer and gives an analyst a way to confirm a positive result on a different principle. Each method carries its own reagents, instruments, analysis procedure, expression of results and precision clauses, and Annex A shows reference chromatograms. Ascorbic acid is added at the very start, with 500 g of the edible portion, because ethoxyquin is itself an antioxidant and begins to degrade once the fruit is crushed and air reaches it - skip that step and the result comes out low and the consignment looks compliant when it is not. For food testing laboratories, fruit importers and packers, and market regulators.

This Standard specifies the liquid chromatography and gas chromatography determination methods for ethoxyquin in food.

This Standard applies to the determination of ethoxyquin in fresh fruits.

Method I: Liquid chromatography

2 Principle

The ethoxyquin in the sample is extracted with n-hexane under alkaline conditions, blown to dryness with nitrogen, redissolved in acetonitrile solution containing ascorbic acid, determined by liquid chromatography, and quantified by external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical regent, and the water is Grade 1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Sodium hydroxide (NaOH).

3.1.2 Ascorbic acid (C6H8O6).

3.1.3 Acetonitrile (C2H3N): chromatographically pure.

3.1.4 n-hexane (C6H14).

3.2 Preparation of reagents

3.2.1 Sodium hydroxide solution (0.1 mol/L): Weigh 4 g of sodium hydroxide, dissolve in water and dilute to 1000 mL.

3.2.2 Acetonitrile solution containing ascorbic acid: Weigh 0.1 g of ascorbic acid, dissolve in 100 mL of acetonitrile, shake thoroughly, filter, and take the filtrate. Prepare freshly each time before use.

3.3 Reference material

Ethoxyquin reference material (C14H19NO, CAS number: 91-53-2): purity >= 99 %, or a reference material certified by the country and awarded a reference material certificate.

3.4 Preparation of standard solutions

3.4.1 Ethoxyquin standard stock solution (1.00 mg/mL): Weigh 100 mg of ethoxyquin reference material (accurate to 0.1 mg) in a 100 mL volumetric flask, dissolve with acetonitrile solution containing ascorbic acid and dilute to the mark, and mix well. Transfer the solution to a brown glass container and store it in the dark at -18 °C. The validity period is 3 months.

3.4.2 Ethoxyquin standard intermediate solution (10.0 ug/mL): Pipette 1.00 mL of the standard stock solution (1.00 mg/mL) into a 100 mL volumetric flask, dilute to the mark with acetonitrile solution containing ascorbic acid, and mix well. Transfer the solution to a brown glass container and store it in the dark at -18 °C. The validity period is 1 month.

3.4.3 Ethoxyquin standard use solution (1.00 ug/mL): Pipette 1.00 mL of the standard intermediate solution (10.0 ug/mL) into a 10 mL volumetric flask, dilute to the mark with acetonitrile solution containing ascorbic acid, and mix well. Prepare freshly each time before use.

3.4.4 Ethoxyquin standard series working solutions: Pipette 40.0 uL, 100 uL, 200 uL, 500 uL and 1000 uL of the standard use solution (1.00 ug/mL) into 10 mL volumetric flasks respectively, dilute to the mark with acetonitrile solution containing ascorbic acid, and mix well. The concentrations of ethoxyquin standard series working solutions are 4.00 ng/mL, 10.0 ng/mL, 20.0 ng/mL, 50.0 ng/mL and 100 ng/mL respectively. Prepare freshly each time before use.

4 Instruments and equipment

4.1 Liquid chromatograph: equipped with a fluorescence detector.

4.2 Tissue masher.

4.3 Analytical balances: the minimum divisions are 0.01 g and 0.1 mg respectively.

4.4 Vortex mixer.

4.5 Oscillator.

4.6 Nitrogen concentration device.

4.7 Centrifuge.

5 Analysis procedure

5.1 Preparation of samples

Take 500 g of the edible part of the representative sample, add 10 g of ascorbic acid, crush and mix with a tissue masher and process into a slurry, put into a clean container and seal it. The prepared samples are frozen and stored at -18 °C for determination as soon as possible.

NOTE: When GB 2763 is used for judgment, the determination site shall be in accordance with the

provisions of GB 2763.

5.2 Extraction of samples

Weigh 5 g (accurate to 0.01 g) of the sample in a 50 mL centrifuge tube, add 5 mL of sodium hydroxide solution (0.1 mol/L), vortex and mix for 30 s, add 10 mL of n-hexane, vortex and mix for 30 s, oscillate for 15 min for extraction, then centrifuge at 4000 r/min for 5 min, transfer the n-hexane layer to a 25 mL volumetric flask, add 10 mL n-hexane to the residue, repeat the extraction once, centrifuge at 4000 r/min for 5 min, combine the n-hexane layer in the same volumetric flask, add n-hexane to dilute to the mark, and mix well. Pipette 5.0 mL of n-hexane extract, concentrate to dryness with nitrogen at 30 °C, quickly add 5.0 mL of acetonitrile solution containing ascorbic acid, vortex and mix for 30 s, filter through a 0.45 um organic microporous membrane to prepare the sample solution to be tested.

5.3 Reference conditions for liquid chromatography

a) Chromatographic column: C18 chromatographic column (4.6 mm x 250 mm, 5 um) or one with equivalent performance.

b) Mobile phase: acetonitrile-water (65 : 35, volume ratio).