SN

ENTRY AND EXIT INSPECTION AND QUARANTINE INDUSTRY

STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 0869-2017

Replacing SN/T 0869-2000

Determination of ascorbic acid in beverage for export

出口饮料中抗坏血酸的测定

Issued on: May 12, 2017 **Implemented on: December 01, 2017**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine of PRC

Table of Contents

Determination of ascorbic acid in beverage for export

1 Scope

The standard specifies the method for the determination of ascorbic acid in beverages for export, by high performance liquid chromatography and fluorescence method.

The first method of this standard is applicable to the determination of total L(+)-ascorbic acid and D(+)-ascorbic acid in beverages for export. The second method is applicable to the determination of the total amount of L(+)-ascorbic acid in beverages for export.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

3 Terms and definitions

The following terms and definitions apply to this document.

3.1

Ascorbic acid

An acidic polyhydroxyl compound, which contains 6 carbon atoms AND is divided into L type and D type.

3.2

L(+)-ascorbic acid

Left-handed and right-handed optical ascorbic acid. It has strong reducing property AND has biological activity on human body.

3.3

D(+)-ascorbic acid

5.6 Hexadecyl trimethyl iron bromide ($C_{16}H_{42}BrN$): Chromatographically pure.

5.7 Methanol: Chromatographically pure.

5.8 Metaphosphoric acid solution (200 g/L): Weigh 200 g (accurate to 0.1 g) of metaphosphoric acid (5.1). Dissolve it in water and dilute to 1 L. This solution can be stored at 4 °C for one month.

5.9 Metaphosphoric acid solution (20 g/L): Take 50 mL of 200 g/L metaphosphoric acid solution. Use water to dilute it to 500 mL.

5.10 Trisodium phosphate solution (100 g/L): Weigh 100 g (accurate to 0.1 g) of trisodium phosphate. Use water to dissolve it to 1 L.

5.11 L-cysteine solution (40 g/L): Weigh 20 g of L-cysteine. Dissolve in water and dilute to 500 mL. Prepare immediately before use.

5.12 L(+)-ascorbic acid standard (CAS No.: 50-81-7): purity ≥ 99%.

5.13 D(+)-ascorbic acid standard (CAS No.: 89-65-6): purity ≥ 99%.

5.14 L(+)-ascorbic acid standard stock solution (1.000 mg/mL): Accurately weigh 0.01 g of L(+)-ascorbic acid standard substance (accurate to 0.01 mg). Use 20 g/L metaphosphoric acid solution, dilute it to 10 mL. The stock solution can be stored for one week, at 2 °C ~ 8 °C in the dark.

5.15 D(+)-ascorbic acid standard stock solution (1.000 mg/mL): Accurately weigh 0.01 g of D(+)-ascorbic acid standard product (accurate to 0.01 mg). Use 20 g/L metaphosphoric acid solution, dilute to 10 mL. The stock solution can be stored for one week, at 2 °C ~ 8 °C in the dark.

5.16 Ascorbic acid mixed standard series working solution: Pipette 0 mL, 0.05 mL, 0.50 mL, 1.0 mL, 2.5 mL, 5.0 mL, respectively, of L(+)-ascorbic acid and D(+)-ascorbic acid standard stock solution. Use 20 g/L metaphosphoric acid solution to dilute it to100 mL. The concentrations of L(+)-ascorbic acid and D(+)-ascorbic acid, in the standard series of working solutions, are 0 μg/mL, 0.5 μg/mL, 5.0 μg/mL, 10.0 μg/mL, 25.0 μg/mL, 50.0 μg/mL, respectively. Store it in the dark. The storage time is 24 h.

6 Instruments and apparatuses

6.1 High-performance liquid chromatography: Equipped with a diode array detector or an ultraviolet detector.

6.2 pH meter: The accuracy is 0.01.

6.3 Balance: Sensitivity is 0.1 g, 1 mg, 0.01 mg.

6.4 Ultrasonic cleaner.

6.5 Centrifuge: Speed ≥ 4000 r/min.

6.6 Homogenizer.

6.7 Filter membrane: 0.45 μm aqueous phase membrane.

6.8 Oscillators.

7 Specimen preparation and storage

7.1 Specimen preparation

Solid or semi-solid samples are crushed and mixed by a tissue masher. The liquid samples are mixed evenly. After mixing, the samples are placed in an airtight container, then prepare for weighing. The test shall be completed, as soon as possible after the sample is prepared.

7.2 Specimen storage

The specimen is stored in airtight storage at 0 ° C ~ 4 ° C. During the operation of specimen preparation and sample storage, it shall avoid the sample from exposure in a large amount of air for a long time, so as to prevent the content of the analyte from change, due to the analyte in the sample being oxidized.

8 Analytical procedures

8.1 Extraction

Weigh about 1 g (accurate to 0.01 g) of solid specimen, OR measure 5 mL ± 0.05 mL of liquid specimen in a 50 mL beaker. Use metaphosphoric acid solution (5.9), then transfer the specimen into a 50 mL volumetric flask. Shake to dissolve and make its volume reach to the mark. Shake well. Transfer all into a 50 mL centrifuge tube. After ultrasonic extraction for 5 min, centrifuge at 4000 r/min for 5 min. Accurately absorb 20 mL of the above centrifuged supernatant, into a 50 mL centrifuge tube. Add 10 mL of L-cysteine solution (5.11). Use trisodium phosphate solution (5.10), then adjust the pH to 7.0 ~ 7.2. Shake for 5 minutes. Use phosphoric acid, then adjust the pH to 2.5 ~ 2.8. Transfer all the test solution into a 50 mL volumetric flask. Use water dilute it to the mark. After mixing, pass it through a 0.45 μm aqueous phase filter membrane, for HPLC determination.

Note: The whole detection process shall be carried out under dark conditions as much as possible.

8.2 Determination by liquid chromatography

Where:

X - The total amount of L(+)-ascorbic acid [or D(+)-ascorbic acid) in the specimen, in milligrams per hectogram (mg/100 g);

c_1 - The measured value of the total amount of L(+)-ascorbic acid [or D(+)-ascorbic acid] in the specimen liquid, in microgram per milliliter (μg/mL);

c_0 - The measured value of the total amount of L(+)-ascorbic acid [or D(+)-ascorbic acid] in the specimen blank solution, in microgram per milliliter (μg/mL);

V - The final constant volume of the specimen, in milliliters (mL);

m - The actual mass of test specimen, in grams (g);

1000 - Conversion factor from μg/mL to mg/mL;

F - Dilution factor (2.5);

100 - Conversion factor from mg/g to mg/100 g.

10 Limit of quantitation and recovery rate

10.1 Limit of quantitation

The limit of quantification (LOQ) of the total amount of L(+)-ascorbic acid and D(+)-ascorbic acid, in this method, is 0.4 mg/100 g.

10.2 Recovery rate

The recoveries are as shown in Table 1.

12.3 Sulfuric acid (H_2SO_4): The concentration is about 98%.

12.4 Sodium acetate (CH_3COONa).

12.5 Boric acid (H_3BO_3).

12.6 o-Phenylenediamine ($C_6H_8N_2$).

12.7 Thymol blue ($C_{27}H_{30}O_5S$).

12.8 Activated carbon powder.

12.9 Metaphosphoric acid-acetic acid solution: Weigh 15 g of metaphosphoric acid. Add 40 mL of glacial acetic acid and 250 mL of water. Heat and stir to dissolve it gradually. Then add water to 500 mL, after cooling. It can be stored in the refrigerator at 4 °C for 7 ~ 10 days.

12.10 Sulfuric acid solution (0.15 mol/L): Take 8.3 mL of sulfuric acid. Carefully add it to water. Then use water to dilute to 1000 mL.

12.11 Metaphosphoric acid-acetic acid-sulfuric acid solution: Weigh 15 g of metaphosphoric acid. Add 40 mL of glacial acetic acid. Add dropwise 0.15 mol/L sulfuric acid solution until dissolved. Dilute to 500 mL.

12.12 Sodium acetate solution (500 g/L): Weigh 500 g of sodium acetate. Add water to 1000 mL.

12.13 Boric acid-sodium acetate solution: Weigh 3 g of boric acid. Use 500 g/L sodium acetate solution to dissolve it AND dilute it to 100 mL. Prepare just before use.

12.14 O-phenylenediamine solution (200 mg/L): Weigh 20 mg of o-phenylenediamine. Use water to dissolve and dilute it to 100 mL. Prepare it immediately before use.

12.15 Acidic activated carbon: Weigh about 200 g of activated carbon powder (75 μm ~ 177 μm). Add 1 L of hydrochloric acid (1 + 9). Heat for back-flow for 1 h ~ 2 h. Filter it. Use water to wash, until there is no iron ion in the filtrate. Place it in an oven, at 110 °C ~ 120 °C for 10 h. Prepare for use.

12.16 Method for testing iron ions: Use Prussian blue reaction. Mix 20 g/L potassium ferrocyanide and 1% hydrochloric acid, in equal amounts. Add the above-mentioned eluted filtrate dropwise. If there are iron ions, a blue precipitate will be produced.

12.17 Thymol blue indicator solution (0.4 mg/mL): Weigh 0.1 g of thymol blue. Add about 10.75 mL of 0.02 mol/L sodium hydroxide solution. Grind in a glass mortar until dissolved. Use water to dilute it to 250 mL. (Discoloration range: red when the pH is equal to 1.2; yellow when the pH is equal to 2.8; blue when the pH is greater than 4).

12.18 L(+)-ascorbic acid standard ($C_6H_8O_6$): purity ≥ 99%.

12.19 L(+)-ascorbic acid standard solution (1.000 mg/mL): Weigh 0.05 g of L(+)-ascorbic acid (accurate to 0.01 mg). Use metaphosphoric acid-acetic acid solution to dissolve and dilute it to 50 mL. The stock solution can be stored for one week, at 2 °C ~ 8 °C in the dark.

12.20 L(+)-ascorbic acid standard working solution (100.0 μg/mL): Pipette 10.00 mL of L(+)-ascorbic acid standard solution. Use metaphosphoric acid-acetic acid solution to dilute it to 100 mL. Prepare immediately before use.

13 Instruments and apparatuses

Fluorescence spectrophotometer: It has an excitation wavelength of 338 nm and an emission wavelength of 420 nm, equipped with a 1 cm cuvette.

14 Analytical procedures

14.1 Extraction

Weigh about 100 g (accurate to 0.1 g) of the specimen. Add 100 g of metaphosphoric acid-acetic acid solution. Pour it into a masher to make a homogenate. Use a thymol blue indicator to test the pH of the homogenate. If it is red, take an appropriate amount of homogenate. Use metaphosphoric acid-acetic acid solution to dilute it. If it is yellow or blue, weigh an appropriate amount of homogenate; use metaphosphoric acid-acetic acid-sulfuric acid solution to dilute it to make the pH at 1.2. The amount of homogenate is determined, according to the content of ascorbic acid in the specimen. When the ascorbic acid content in the specimen solution is between 40 μg/mL ~ 100 μg/mL, generally weigh 20 g (accurate to 0.01 g) of the homogenate; use corresponding solution to dilute it to 100 mL. Filter it. Take the filtrate for later use.

14.2 Determination

14.2.1 Oxidation treatment: Accurately pipette 50 mL of the specimen filtrate and ascorbic acid standard working solution, into a 200 mL conical flask with a stopper. Add 2 g of activated carbon. Shake vigorously for 1 min. Filter it. Discard the initial several milliliters of filtrate. Collect the rest of the filtrate respectively, that is, get the specimen oxidation solution and the standard oxidation solution. Prepare for determination.

14.2.2 Accurately pipette 10 mL of specimen oxidation solution, into two 100 mL volumetric flasks, respectively, which are "specimen solution" and "specimen blank solution", respectively.

14.2.3 Accurately pipette 10 mL of standard oxidation solution, into two 100 mL volumetric flasks, respectively, which are "standard solution" and "standard blank solution".

This is an excerpt of the PDF (Some pages are marked off intentionally)

------ The End ------