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National Food Safety Standard - Determination of Cyanide in Foods

食品安全国家标准　食品中氰化物的测定

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Table of Contents

Foreword 4
1 Scope 5
Method I - Spectrophotometry 5
2 Principle 5
3 Reagents and Materials 5
4 Instruments and Equipment 7
5 Analytical Procedures 7
6 Expression of Analysis Results 10
7 Precision 12
8 Others 12
Method II - Gas Chromatography 12
9 Principle 12
10 Reagents and Materials 13
11 Instruments and Equipment 14
12 Analytical Procedures 14
13 Expression of Analysis Results 16
14 Precision 17
15 Others 17
Method III - Gas Chromatography - Mass Spectrometry 17
16 Principle 17
17 Reagents and Materials 18
18 Instruments and Equipment 18
19 Analytical Procedures 18
20 Expression of Analysis Results 20
21 Precision 20
22 Others 20
Method IV - Ion Chromatography 20
23 Principle 20
24 Reagents and Materials 20
25 Instruments and Equipment 22
26 Analytical Procedures 22

27 Expression of Analysis Results 24
28 Precision 24
29 Others 24
Method V - Flow Injection / Continuous Flow - Spectrophotometry 25
30 Principle 25
31 Reagents and Materials 25
32 Instruments and Equipment 27
33 Analytical Procedures 27
34 Expression of Analysis Results 28
35 Precision 28
36 Others 29
Appendix A Reference Conditions of Headspace Analysis 30
Appendix B Standard Gas Chromatogram of Cyanogen Chloride 31
Appendix C Setting of Ion Chromatography Conditions and Examples of Standard Chromatograms 33
Appendix D Cyanide Ion Determination Chart of Distilled Liquor and Its Integrated Alcoholic Beverages under Flow Injection / Continuous Flow 35

National Food Safety Standard - Determination of Cyanide in Foods

1 Scope

This Standard specifies the method for the determination of cyanide in foods.

The first method “spectrophotometry” is applicable to the determination of cyanide in distilled liquor and its integrated alcoholic beverages, edible alcohol, tapioca flour, packaged drinking water and mineral water.

The second method “gas chromatography” and the third method “gas chromatography - mass spectrometry” are applicable to the determination of cyanide in liquor, edible alcohol, grain, packaged drinking water and mineral water.

The fourth method “ion chromatography” is applicable to the determination of cyanide in distilled liquor and its integrated alcoholic beverages, edible alcohol, packaged drinking water, mineral water and beverages (based on almonds).

The fifth method “flow injection / continuous flow - spectrophotometry” is applicable to the determination of cyanide in distilled liquor and its integrated alcoholic beverages, edible alcohol, packaged drinking water and mineral water.

Method I - Spectrophotometry

2 Principle

Heat the specimen under alkaline conditions to remove high-boiling-point organic matters, or heat and distill it under acidic conditions. After using sodium hydroxide solution to dissolve or absorb it, under the condition of pH = 7.0, use chloramine T to convert cyanide to cyanogen chloride, then, react it with isonicotinic acid-pyrazolone to generate a blue dye, which can be quantitatively compared with the standard series.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-3 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Absolute ethanol (C_2H_6O).

distillation device (for water cooling, a condenser is required to adjust the temperature to 10 °C ~ 15 °C for distillation), insert the lower end of the condenser tube under the liquid level of a 100 mL volumetric flask containing 10 mL of sodium hydroxide solution (20 g/L) (if the room temperature is higher than 25 °C, use an ice pack to cool it down, so that the temperature of the distillate is between 20 °C ~ 25 °C), and perform distillation. When the distillate collected is nearly 100 mL, use water to reach a constant volume to the scale. Accurately draw 1.00 mL of the solution into a 10 mL colorimetric tube, add 2 g/L of sodium hydroxide solution to 5 mL, shake it well, and use it as a specimen solution.

5.1.1.2 Add 2 drops of phenolphthalein indicator solution to the specimen solution, add acetic acid solution to adjust it, until the red color fades, then, use 20 g/L sodium hydroxide solution to adjust it to nearly red color. Add 2 mL of phosphate buffer solution, then add 0.2 mL of chloramine T solution, shake it well and let it stand for 3 min. Add 2 mL of isonicotinic acid-pyrazolone solution, add water to reach a constant volume to the scale. Add a stopper and shake to evenly mix it. Place it in a constant-temperature water bath at 37 °C for 40 min and reserve it for testing.

5.1.2 Distilled liquor and its integrated alcoholic beverages, edible alcohol

5.1.2.1 Accurately draw 1.00 mL of specimen (for sesame-flavor liquor, Maotai-flavor liquor and special-flavor liquor specimens, draw 0.10 mL) into a 25 mL beaker, add 5 mL of sodium hydroxide solution (2 g/L), and leave it for 10 min. Then, place it on an electric heating plate at 160 °C ~ 180 °C and heat it at low temperature, until about 1 mL of the solution remains. Remove it and place it at room temperature. Use 2 g/L sodium hydroxide solution to transfer it to a 10 mL colorimetric tube with a stopper. Finally, add 2 g/L sodium hydroxide solution to 5 mL.

5.1.2.2 If the liquor specimen is turbid or colored, take 25.0 mL of specimen into a 250 mL distillation flask, add 100 mL of water, add a few glass beads, and dropwise add a few drops of methyl orange indicator. Insert the lower end of the condenser tube under the liquid level of the colorimetric tube containing 10 mL of sodium hydroxide solution (20 g/L), add 1 g ~ 2 g of tartaric acid, the solution changes from orange to orange-red. Quickly connect the distillation device to perform distillation, and collect nearly 50 mL of the distillate, then, use water to reach a constant water of 50 mL, and evenly mix it. Take 2.00 mL of the distillate and operate in accordance with 5.1.2.1.

5.1.2.3 Same as 5.1.1.2.

5.1.3 Packaged drinking water and mineral water

5.1.3.1 Measure-take 250 mL of water specimen (when the cyanide content exceeds 20 μg, take an appropriate amount of water specimen and add water to 250 mL) and place it into a 500 mL distillation device. Add 1 ~ 2 drops of methyl orange indicator, then, add 5 mL of zinc acetate solution, and add 1 g ~ 2 g of tartaric acid, the solution changes from orange to orange-red. Quickly connect the distillation device, insert the lower end of the condenser tube under the liquid level of a 50 mL colorimetric tube with a stopper containing 5.0 mL of sodium hydroxide

solution (20 g/L). By adjusting the temperature, control the distillation speed at 2 mL/min ~ 3 mL/min, collect nearly 50 mL of the distillate, then, use water to reach a constant water of 50 mL, and evenly mix it. Take 10.0 mL of the distillate and place it in a 25 mL colorimetric tube with a stopper.

5.1.3.2 Add 5.0 mL of phosphate buffer solution to the colorimetric tube containing the specimen, place it in a constant-temperature water bath at 37 °C, then, add 0.25 mL of chloramine T solution, add a stopper to mix it, leave it for 5 min. Then, add 5.0 mL of isonicotinic acid-pyrazolone solution, add water to 25 mL and mix it well. Place it in a constant-temperature water bath at 37 °C for 40 min and reserve it for testing.

5.2 Drawing of Standard Working Curve

5.2.1 Standard curve of tapioca flour

Respectively and accurately draw 0 mL, 0.40 mL, 0.80 mL, 1.20 mL, 1.60 mL and 2.00 mL of cyanide ion standard solution (1.0 μg/mL) into a 10 mL colorimetric tube [equivalent to the mass of cyanide (CN^-): 0 μg, 0.40 μg, 0.80 μg, 1.20 μg, 1.60 μg and 2.00 μg], add 2 g/L sodium hydroxide solution to 5 mL. In accordance with 5.1.1.2, conduct synchronous operation with the sample below. Take the prepared standard curve solution to be tested, use a 1 cm cuvette, and take 2 g/L sodium hydroxide solution as the blank solution to adjust the zero point. At a wavelength of 638 nm, determine the absorbance; take the mass of cyanide ions as the x-coordinate and the absorbance of cyanide ions as the y-coordinate, and draw a standard curve.

5.2.2 Standard curve of distilled liquor and its integrated alcoholic beverages, edible alcohol

Respectively and accurately draw 0 mL, 0.40 mL, 0.80 mL, 1.20 mL, 1.60 mL and 2.00 mL of cyanide ion standard solution (1.0 μg/mL) into a 50 mL beaker [equivalent to the mass of cyanide (CN^-): 0 μg, 0.40 μg, 0.80 μg, 1.20 μg, 1.60 μg and 2.00 μg], add 5 mL of sodium hydroxide solution (2 g/L) and leave it for 10 min. In accordance with 5.1.2.1 and 5.1.2.3, conduct synchronous operation with the sample below. Take the prepared standard curve solution to be tested, use a 1 cm cuvette, and take 2 g/L sodium hydroxide solution as the blank solution to adjust the zero point. At a wavelength of 638 nm, determine the absorbance; take the mass of cyanide ions as the x-coordinate and the absorbance of cyanide ions as the y-coordinate, and draw a standard curve.

5.2.3 Standard curve of packaged drinking water and mineral water

Respectively and accurately draw 0 mL, 0.10 mL, 0.20 mL, 0.40 mL, 0.80 mL and 1.00 mL of cyanide ion standard solution (1.0 μg/mL) into a 25 mL colorimetric tube [equivalent to the mass of cyanide (CN^-): 0 μg, 0.10 μg, 0.20 μg, 0.40 μg, 0.80 μg and 1.00 μg], add 2 g/L sodium hydroxide solution to 10.0 mL. In accordance with 5.1.3.2, conduct synchronous operation with the sample below. Take the prepared standard curve solution to be tested, use a 3 cm cuvette, and take 2 g/L sodium hydroxide solution as the blank solution to adjust the zero point. At a wavelength of 638 nm, determine the absorbance; take the mass of cyanide ions as the x-coordinate and the absorbance of cyanide ions as the y-coordinate, and draw a standard curve.

cyanide in the specimen, under acidic conditions, use chloramine T to derive it into cyanogen chloride. Cyanogen chloride reaches equilibrium in the gas phase and liquid phase, and the gas phase part is introduced into the gas chromatograph for separation. Use an electron capture detector for detection and the external standard method for quantitative determination.

10 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-2 water specified in GB/T 6682.

10.1 Reagents

10.1.1 Chloramine T ($C_7H_7ClNNaO_2S \bullet 3\ H_2O$).

10.1.2 Phosphoric acid (H_3PO_4): ≥ 85%.

10.1.3 Sodium hydroxide (NaOH).

10.2 Reagent Preparation

10.2.1 Chloramine T solution (10 g/L): weigh-take 0.1 g of chloramine T, use water to dissolve it and reach a constant volume of 10 mL. Prepare it right before use.

10.2.2 Phosphoric acid solution (1 + 5): measure-take 10 mL of concentrated phosphoric acid, add it to 50 mL of water, and evenly mix it.

10.2.3 0.1% sodium hydroxide solution: weigh-take 1.0 g of sodium hydroxide, use water to dissolve it and reach a constant volume of 1 L.

10.2.4 0.01 mol/L sodium hydroxide solution: weigh-take 0.4 g of sodium hydroxide, use water to dissolve it and reach a constant volume of 1 L.

NOTE: when the prepared chloramine T solution is turbid, it needs to be replaced with newly prepared chloramine T.

10.3 Reference Material

Cyanide standard solution (50.0 μg/mL, calculated as CN^-): a standard substance certified by the state and awarded a reference material certificate. Please refer to the reference material certificate for shelf life and storage conditions.

10.4 Preparation of Standard Solutions

10.4.1 Cyanide ion (calculated as CN^-) standard intermediate solution: accurately transfer-take 2.00 mL of cyanide standard solution (50.0 μg/mL) into a 10.0 mL volumetric flask; use 0.1% sodium hydroxide solution to reach a constant volume. The mass concentration of this solution is 10.0 mg/L. Prepare it right before use.

10.4.2 Cyanide ion (calculated as CN^-) standard working solutions: transfer-take an appropriate amount of cyanide ion (calculated as CN^-) standard intermediate solution (10.0 mg/L), and use 0.01 mol/L sodium hydroxide solution to dilute it and prepare working solutions respectively with a mass concentration of 0.001 mg/L, 0.005 mg/L, 0.050 mg/L, 0.100 mg/L and 0.200 mg/L. Prepare it right before use.

NOTE: since cyanide is a highly toxic substance, please operate in a fume hood.

11 Instruments and Equipment

11.1 Gas chromatograph: equipped with an electron capture detector (ECD).

11.2 Headspace sampler.

11.3 Headspace bottle: 20 mL.

11.4 Vortex mixer.

11.5 Analytical balance: with a division value of 0.0001 g.

11.6 Ultrasonic cleaner.

11.7 Centrifuge: with a rotational speed ≥ 4,000 r/min.

12 Analytical Procedures

12.1 Pre-treatment of Sample

12.1.1 Specimen preparation

Take about 500 g of solid specimen, use a sample crushing device to make it into powder, put it into a clean container, seal it, and store it at 0 °C ~ 4 °C. Take about 500 mL of liquid specimen, thoroughly mix it, put it into a clean container, seal it, and store it at 0 °C ~ 4 °C.

NOTE: during the operation of specimen preparation, samples must be prevented from being contaminated.

12.1.2 Determination of specimen solution

12.1.2.1 Liquor and edible alcohol

Accurately transfer-take 0.20 mL of specimen into a headspace bottle, add 9.80 mL of 0.01 mol/L sodium hydroxide solution, let it stand for 5 min, add 0.2 mL of phosphoric acid solution. Then, add 0.2 mL of chloramine T solution, immediately cap seal it, and reserve it for testing.

12.1.2.2 Grain

24.1.4 Methanol (CH_3OH).

24.1.5 Acetonitrile (C_2H_3N).

24.2 Reagent Preparation

24.2.1 Eluent: 100 mmol/L sodium hydroxide + 500 mmol/L sodium acetate + 0.5% ethylenediamine (volume ratio): weigh-take 41.02 g of sodium acetate and dissolve it in 1 L of water; pass it through a 0.22 μm nylon microporous filter membrane, add 5.00 mL of ethylenediamine and 5.24 mL of 50% sodium hydroxide.

24.2.2 500 mmol/L sodium hydroxide solution: draw 2.62 mL of 50% sodium hydroxide, add it to 100 mL of water, and evenly mix it.

24.3 Reference Material

Cyanide standard solution (50.0 μg/mL, calculated as CN^-): a standard substance certified by the state and awarded a reference material certificate. Please refer to the reference material certificate for shelf life and storage conditions.

24.4 Preparation of Standard Solution

24.4.1 Preparation of distilled liquor and its integrated alcoholic beverages, edible alcohol standard series solutions

24.4.1.1 Cyanide (calculated as CN^-) standard intermediate solution (1.00 mg/L): draw 0.20 mL of cyanide standard solution (50.0 μg/mL) into a 10 mL volumetric flask, add water to reach a constant volume to the scale, and evenly mix it. Prepare it right before use.

24.4.1.2 Cyanide (calculated as CN^-) standard series working solutions: respectively draw 0.050 mL, 0.10 mL, 0.20 mL, 0.50 mL and 1.00 mL of cyanide (calculated as CN^-) standard intermediate solution (1.00 mg/L) into a 10 mL volumetric flask, add water to reach a constant volume to the scale, and evenly mix it. The mass concentration of the cyanide (calculated as CN^-) standard series working solutions is respectively 0.0050 mg/L, 0.010 mg/L, 0.020 mg/L, 0.050 mg/L and 0.100 mg/L. Prepare it right before use.

24.4.2 Preparation of packaged drinking water and mineral water standard series solutions

24.4.2.1 Cyanide (calculated as CN^-) standard intermediate solution (1.00 mg/L): draw 0.20 mL of cyanide standard solution (50.0 μg/mL) into a 10 mL volumetric flask, add water to reach a constant volume to the scale, and evenly mix it. Prepare it right before use.

24.4.2.2 Cyanide (calculated as CN^-) standard series working solutions: respectively draw 0.050 mL, 0.10 mL, 0.20 mL, 0.50 mL and 1.00 mL of cyanide standard solution (1.00 mg/L) into a 10 mL volumetric flask, add water to reach a constant volume to the scale. Then, add 0.10 mL of sodium hydroxide solution (500 mmol/L), and evenly mix it. The mass concentration of the cyanide (calculated as CN^-) standard series working solutions is

Take about 500 mL of sample, thoroughly mix it, put it into a clean container, seal it, and store it at 0 °C ~ 4 °C.

26.1.2 Specimen dilution, purification and pH adjustment

26.1.2.1 Packaged drinking water and mineral water

Accurately transfer-take 10.0 mL of specimen into a colorimetric tube, add 0.1 mL of sodium hydroxide solution (500 mmol/L), evenly mix it, and reserve it for testing.

26.1.2.2 Distilled liquor and its integrated alcoholic beverages, edible alcohol

Accurately transfer-take 1.00 mL of specimen into a 10.0 mL colorimetric tube, use water to dilute to reach a constant volume to the scale, and evenly mix it. Then, filter it through 0.22 μm nylon microporous filter membrane and reserve it for testing. When the sample color is relatively dark or the sugar content is relatively high, firstly, pass the test solution through the IC-RP solid-phase extraction column, control the entire flow rate at 4 mL/min, discard the initial 3 mL or 6 mL (selected in accordance with the column specifications), then, filter it through the 0.22 μm nylon microporous filter membrane, and reserve it for testing.

26.1.2.3 Beverages (based on almonds)

Accurately transfer-take 4.00 mL of specimen into a 10.0 mL colorimetric tube, use acetonitrile to reach a constant volume to the scale, perform vortex mix for 2 min. Transfer it to a centrifuge tube, let it stand, then, centrifuge for 10 min. Accurately transfer-take 2.5 mL of the supernatant and use water to reach a constant volume to 10.0 mL. Take an appropriate amount of the above-mentioned solution, firstly, pass it through the IC-RP solid phase extraction column, control the entire flow rate at 4 mL/min, discard the initial 3 mL or 6 mL (selected in accordance with the column specifications), then, filter it through the 0.22 μm nylon microporous filter membrane, and reserve it for testing.

26.2 Reference Conditions of Instruments

Chromatographic column: the filler is an anion protection column and separation column of polystyrene divinylbenzene copolymer with alkyl quaternary ammonium groups, or those with equivalent performance.

Eluent: 100 mmol/L sodium hydroxide + 500 mmol/L sodium acetate + 0.5% ethylenediamine (volume ratio), perform isocratic elution.

Flow rate: 1 mL/min.

Column temperature: 30 °C.

Detector: ampere detector.

Injection volume: 250 μL for beverages (based on almonds); 50 μL for others.

31.2 Reagent Preparation

31.2.1 Flow injection method

31.2.1.1 Sodium hydroxide solution (1.0 g/L): weigh-take 1.0 g of sodium hydroxide, dissolve in water, and reach a constant volume of 1,000 mL. Prepare it right before use. Before use, it needs to be degassed. This solution is also used for the standard curve and the alkaline hydrolysis dilution of liquor samples.

31.2.1.2 Distillation reagent (pH = 3.8): weigh-take 13.2 g of tartaric acid into a 1,000 mL beaker, add 800 mL of water, then, weigh 3.5 g of sodium hydroxide and add it to the above-mentioned solution to dissolve, then, use water to reach a constant volume in a 1,000 mL volumetric flask.

31.2.1.3 Buffer solution (pH = 4.0): weigh-take 97.0 g of MKP (KH_2PO_4), use water to reach a constant volume of 1,000 mL. It shall remain valid for 1 month.

31.2.1.4 Chloramine T solution: weigh-take 2.0 g of chloramine T and reach a constant volume in 500 mL of water. Place it in a brown glass bottle and prepare it right before use. Before use, it needs to be degassed.

31.2.1.5 Isonicotinic acid / barbituric acid color developer: weigh-take 12.0 g of sodium hydroxide, add 500 mL of water to dissolve it, add until it is completely dissolved. Then, weigh-take 16.8 g of 1,3-dimethylbarbituric acid and add to it. After it is completely dissolved, add 13.6 g of isonicotinic acid, use water to reach a constant volume to 1,000 mL, and store it in a brown glass bottle. It shall remain valid for 1 month (if there are insoluble substances in the solution, before use, it needs to be filtered with a filter paper). Before use, it needs to be degassed.

31.2.2 Continuous flow method

31.2.2.1 0.5 mol/L sodium hydroxide solution: weigh-take 20 g of sodium hydroxide and dissolve it in 500 mL of water, then, use water to reach a constant volume of 1,000 mL.

31.2.2.2 Distillation reagent: add 250 mL of phosphoric acid to 500 mL of water, add 50 mL of hypophosphorous acid, evenly mix it, and use water to reach a constant volume of 1,000 mL.

31.2.2.3 0.1 mol/L sodium hydroxide solution: weigh-take 4 g of sodium hydroxide and dissolve it in 500 mL of water, use water to reach a constant volume of 1,000 mL.

31.2.2.4 pH 5.2 buffer solution: dissolve 13.6 g of MKP and 0.34 g of DKP in 800 mL of water, use 0.5 mol/L NaOH to adjust pH = 5.2; use water to reach a constant volume of 1,000 mL, add 3 mL of Brij35 and evenly mix it. Place it in a brown bottle and store in the refrigerator at 4 °C.

31.2.2.5 Chloramine T solution: weigh-take 1 g of chloramine T and dissolve in 500 mL of water.

31.2.2.6 Color developing reagent: weigh-take 4.4 g of sodium hydroxide and dissolve in 400

mL of water, add 6.8 g of barbituric acid and 6.8 g of isonicotinic acid. At 30 °C, stir it for 1 h; use 0.5 mol/L sodium hydroxide solution to adjust pH = 5.5, and reach a constant volume of 500 mL.

31.3 Reference Material

Same as 3.3.

31.4 Preparation of Standard Solution

Same as 3.4.

32 Instruments and Equipment

32.1 Analytical balance: with a division value of 0.001 g.

32.2 pH meter.

32.3 Fully automatic flow injector or flow analyzer: automatic sampler, chemical analysis unit, 600 nm detector and data processing unit.

33 Analytical Procedures

33.1 Pre-treatment of Sample

33.1.1 Packaged drinking water and mineral water

The samples can be directly tested on the machine.

33.1.2 Distilled liquor and its integrated alcoholic beverages, edible alcohol

Accurately draw 1.00 mL of specimen (for sesame-flavor liquor, Maotai-flavor liquor and special-flavor liquor specimens, draw 0.25 mL) into a 25 mL volumetric flask, use sodium hydroxide solution (1.0 g/L) to reach a constant volume to the scale, evenly mix it and let it stand for 10 min. After alkaline hydrolysis, inject the specimen for determination.

33.2 Instrument Operation

Refer to the instrument manual. Turn on the instrument, adjust the flow path system, and select corresponding analysis conditions to preheat the instrument.

33.3 Determination

After the flow path system becomes stable, successively determine the standard series and samples.

33.3.1 Drawing of standard curve

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