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National Food Safety Standard - Determination of Vitamin B_6 in Foods

食品安全国家标准　食品中维生素 B_6 的测定

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National Food Safety Standard - Determination of Vitamin B_6 in Foods

1 Scope

This Standard specifies the method for the determination of vitamin B_6 in foods.

The first method “liquid chromatography - tandem mass spectrometry” is applicable to the determination of vitamin B_6 in foods.

The second method “liquid chromatography - mass spectrometry” is applicable to the determination of vitamin B_6 (pyridoxamine, pyridoxal and pyridoxine) in formulated milk powder, special dietary foods, ready-to-eat cereals, baked goods and beverages, in which, vitamin B_6 (pyridoxamine, pyridoxal and pyridoxine) is added as a nutritional fortifier.

The third method “high-performance liquid chromatography - fluorescence detection method” is applicable to the determination of vitamin B_6 (pyridoxamine, pyridoxal and pyridoxine) in formulated milk powder, special dietary foods (except foods for special medical purposes), ready-to-eat cereals, baked goods and beverages, in which, vitamin B_6 (pyridoxamine, pyridoxal and pyridoxine) is added as a nutritional fortifier.

The fourth method “microbiological method” is applicable to the determination of vitamin B_6 in foods.

Method I - Liquid Chromatography - Tandem Mass Spectrometry

2 Principle

Vitamin B_6 (pyridoxamine, pyridoxal and pyridoxine) in foods is firstly hydrolyzed by acid, then, enzymatically hydrolyzed into pyridoxamine, pyridoxal and pyridoxine. After dilution and filtration, reversed-phase liquid chromatography separation, tandem mass spectrometry detection, and isotope internal standard method for quantitative determination, the total vitamin B_6 content is calculated in terms of pyridoxine.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

3.3.6 D_3-pyridoxamine dihydrochloride ($C_8D_3H_{11}Cl_2N_2O_2$, CAS No.: 1173023-45-4): purity ≥ 98%.

3.4 Preparation of Standard Solutions

3.4.1 Pyridoxine standard stock solution (1.00 mg/mL): accurately weigh-take 60.8 mg of pyridoxine hydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it and dilute into a 50 mL brown volumetric flask. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

3.4.2 Pyridoxal standard stock solution (1.00 mg/mL): accurately weigh-take 60.9 mg of pyridoxal hydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it and dilute into a 50 mL brown volumetric flask. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

3.4.3 Pyridoxamine standard stock solution (1.00 mg/mL): accurately weigh-take 71.7 mg of pyridoxamine dihydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it and dilute into a 50 mL brown volumetric flask. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

3.4.4 Vitamin B_6 standard mixed stock solution (50.0 μg/mL): respectively and accurately draw 5.00 mL of pyridoxamine, pyridoxal and pyridoxine standard stock solution (1.00 mg/mL), use 0.1 mol/L hydrochloric acid solution to dilute into a 100 mL brown volumetric flask. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

3.4.5 Vitamin B_6 standard mixed working solution (5.00 μg/mL): accurately draw 1.00 mL of vitamin B_6 standard mixed stock solution (50.0 μg/mL), use mobile phase A to dilute into a 10 mL brown volumetric flask. Prepare it right before use.

3.4.6 Vitamin B_6 standard mixed working solution (500 ng/mL): accurately draw 1.00 mL of vitamin B_6 standard mixed stock solution (5.00 μg/mL), use mobile phase A to dilute into a 10 mL brown volumetric flask. Prepare it right before use.

3.4.7 Vitamin B_6 standard mixed working solution (50.0 ng/mL): accurately draw 1.00 mL of vitamin B_6 standard mixed stock solution (500 ng/mL), use mobile phase A to dilute into a 10 mL brown volumetric flask. Prepare it right before use.

NOTE: the frozen stock solution shall be thawed at room temperature and evenly mixed before use.

3.5 Preparation of Isotope Internal Standard Solutions

3.5.1 $^{13}C_4$-pyridoxine isotope internal standard stock solution (100 μg/mL): accurately weigh-take 12.1 mg (accurate to 0.1 mg) of $^{13}C_4$-pyridoxine hydrochloride, use 0.1 mol/L hydrochloric acid solution to dilute into a 100 mL brown volumetric flask. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

4.8 Homogenizer.

4.9 Constant-temperature water bath or autoclave.

5 Analytical Procedures

5.1 Specimen Preparation

For meat, vegetables and fruits, etc., take the edible parts, use water to wash them, and use dry gauze to wipe off the surface moisture, use a homogenizer to homogenize them and store in sample bottles for later use. For powdery samples (milk powder and rice noodles, etc.), evenly mix them and conduct direct sampling. For liquid samples, evenly shake them and reserve them for later use. For liquid samples with granules, for example, large-grained yogurt, use a homogenizer to homogenize them and reserve them for later use. For flaky and granular samples, use a high-speed pulverizer to grind them into powder and seal for later use. The prepared specimens shall be kept refrigerated in the dark and determined as soon as possible.

5.2 Specimen Treatment

5.2.1 Specimens containing starch

5.2.1.1 Solid specimens: accurately weigh-take 1 g ~ 5 g (accurate to 0.01 g) of evenly mixed solid specimen into a 150 mL stoppered conical flask (with a soft stopper), and add 500 μL of vitamin B_6 isotope internal standard mixed working solution (10.0 μg/mL), add 50 mL of 0.1 mol/L HCl solution to disperse the sample, use a soft stopper to plug it. Place it in 100 °C water bath or 121 °C autoclave to perform hydrolysis for 30 min; cool to room temperature and take it out. After using sodium hydroxide solution (0.1 mol/L) to adjust the pH to 4.2 ~ 4.8, accurately add 20 mg of acid phosphatase, 100 mg of papain and 10 mg of α-amylase. Fill the conical flask with nitrogen, plug the flask and thoroughly mix it. In a constant-temperature incubator at 37 °C, conduct enzymatic hydrolysis for above 18 h. After the solution cools to room temperature, transfer it to a 100 mL brown volumetric flask. Use water to dilute to the scale and evenly mix it.

5.2.1.2 Liquid specimens: accurately weigh-take 5 g ~ 20 g (accurate to 0.01 g) of evenly mixed liquid specimen into a 150 mL stoppered conical flask (with a soft stopper), and add 500 μL of vitamin B_6 isotope internal standard mixed working solution (10.0 μg/mL), add 50 mL of 0.1 mol/L HCl solution to disperse the sample, use a soft stopper to plug it. Place it in 100 °C water bath or 121 °C autoclave to perform hydrolysis for 30 min; cool to room temperature and take it out. After using sodium hydroxide solution (0.1 mol/L) to adjust the pH to 4.2 ~ 4.8, accurately add 20 mg of acid phosphatase, 100 mg of papain and 10 mg of α-amylase. Fill the conical flask with nitrogen, plug the flask and thoroughly mix it. In a constant-temperature incubator at 37 °C, conduct enzymatic hydrolysis for above 18 h. After the solution cools to room temperature, transfer it to a 100 mL brown volumetric flask. Use water to dilute to the scale and evenly mix it.

5.2.2 Starch-free specimens

5.2.2.1 Solid specimens: weigh-take 1 g ~ 5 g (accurate to 0.01 g) of evenly mixed solid specimen into a 150 mL stoppered conical flask (with a soft stopper), and add 500 μL of vitamin B_6 isotope internal standard mixed working solution (10.0 μg/mL), add 50 mL of 0.1 mol/L HCl solution to disperse the sample, use a soft stopper to plug it. Place it in 100 °C water bath or 121 °C autoclave to perform hydrolysis for 30 min; cool to room temperature and take it out. After using sodium hydroxide solution (0.1 mol/L) to adjust the pH to 4.2 ~ 4.8, accurately add 20 mg of acid phosphatase and 100 mg of papain. Fill the conical flask with nitrogen, plug the flask and thoroughly mix it. In a constant-temperature incubator at 37 °C, conduct enzymatic hydrolysis for above 18 h. After the solution cools to room temperature, transfer it to a 100 mL brown volumetric flask. Use water to dilute to the scale and evenly mix it.

5.2.2.2 Liquid specimens: weigh-take 5 g ~ 20 g (accurate to 0.01 g) of evenly mixed liquid specimen into a 150 mL stoppered conical flask (with a soft stopper), and add 500 μL of vitamin B_6 isotope internal standard mixed working solution (10.0 μg/mL), add 50 mL of 0.1 mol/L HCl solution to disperse the sample, use a soft stopper to plug it. Place it in 100 °C water bath or 121 °C autoclave to perform hydrolysis for 30 min; cool to room temperature and take it out. After using sodium hydroxide solution (0.1 mol/L) to adjust the pH to 4.2 ~ 4.8, accurately add 20 mg of acid phosphatase and 100 mg of papain. Fill the conical flask with nitrogen, plug the flask and thoroughly mix it. In a constant-temperature incubator at 37 °C, conduct enzymatic hydrolysis for above 18 h. After the solution cools to room temperature, transfer it to a 100 mL brown volumetric flask. Use water to dilute to the scale and evenly mix it.

NOTE 1: the operator may adjust the weighing amount of the specimen and the amount of isotope internal standard added in accordance with the vitamin B_6 content of the specimen and the sensitivity of the mass spectrometer in this laboratory, and under conditions that are not lower than the determination range requirements of the standard curve.

NOTE 2: to determine the products in which, vitamin B_6 (pyridoxamine, pyridoxal and pyridoxine) is added as a nutritional fortifier, please refer to 12.2 for the pre-treatment method.

5.2.3 Preparation of test solution

Take another 50 mL conical flask, put in a funnel and filter paper, evenly mix the diluted sample solution and pour the solution into the flask. Naturally filter it to obtain more than 20 mL of filtrate. Accurately draw 1.00 mL of the filtrate into a 10 mL brown volumetric flask, use mobile phase A to dilute to the scale. After vortex mixing, use 0.22 μm microporous membrane to filter it, and transfer it to a brown sample injection bottle for later testing.

5.3 Determination Conditions of Instruments

5.3.1 Reference conditions of chromatography

The reference conditions of chromatography are as follows:

a) Chromatographic column: silica gel matrix pentafluorophenyl column (particle size 1.8 μm, 3.0 mm × 150 mm), or equivalent;

10.1.2 Formic acid (HCOOH): chromatographically pure.

10.1.3 Ammonium formate ($HCOONH_4$): chromatographically pure.

10.1.4 Hydrochloric acid (HCl).

10.1.5 Sodium hydroxide (NaOH).

10.1.6 α-amylase: enzyme activity ≥ 50 U/mg.

10.2 Reagent Preparation

10.2.1 Hydrochloric acid solution (0.1 mol/L): accurately draw 9 mL of hydrochloric acid and use water to dilute to 1,000 mL.

10.2.2 Sodium hydroxide solution (0.1 mol/L): accurately weigh-take 0.4 g of sodium hydroxide, add 50 mL of water to dissolve it; after cooling, use water to dilute to 100 mL.

10.2.3 Mobile phase A (2% formic acid aqueous solution of 10 mmol/L ammonium formate): weigh-take 0.63 g of ammonium formate, successively add about 950 mL of water to dissolve it, accurately add 20 mL of formic acid, and use water to dilute to 1,000 mL. Then, evenly mix it, conduct ultrasonic degassing and reserve it for later use.

10.2.4 Mobile phase B (0.1% formic acid - methanol solution): draw 1 mL of formic acid, use methanol to dilute to 1,000 mL, and conduct ultrasonic mixing.

10.3 Reference Materials

10.3.1 Pyridoxine hydrochloride ($C_8H_{12}ClNO_3$, CAS No.: 58-56-0): purity ≥ 98%, or a standard substance certified by the state and awarded a reference material certificate.

10.3.2 Pyridoxal hydrochloride ($C_8H_{10}ClNO_3$, CAS No.: 65-22-5): purity ≥ 99%, or a standard substance certified by the state and awarded a reference material certificate.

10.3.3 Pyridoxamine dihydrochloride ($C_8H_{14}Cl_2N_2O_2$, CAS No.: 524-36-7): purity ≥ 99%, or a standard substance certified by the state and awarded a reference material certificate.

10.3.4 $^{13}C_4$-pyridoxine hydrochloride ($^{13}C_4C_4H_{12}ClNO_3$): purity ≥ 98%.

10.3.5 D_3-pyridoxal ($C_8D_3H_6NO_3$, CAS No.: 1173023-49-8): purity ≥ 98%.

10.3.6 D_3-pyridoxamine dihydrochloride ($C_8D_3H_{11}Cl_2N_2O_2$, CAS No.: 1173023-45-4): purity ≥ 98%.

10.4 Preparation of Standard Solutions

10.4.1 Pyridoxine standard stock solution (1.00 mg/mL): accurately weigh-take 60.8 mg of pyridoxine hydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it, transfer into a 50 mL brown volumetric flask, and dilute to the scale. Then, transfer

it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

10.4.2 Pyridoxal standard stock solution (1.00 mg/mL): accurately weigh-take 60.9 mg of pyridoxal hydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it, transfer into a 50 mL brown volumetric flask, and dilute to the scale. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

10.4.3 Pyridoxamine standard stock solution (1.00 mg/mL): accurately weigh-take 71.7 mg of pyridoxamine dihydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it, transfer into a 50 mL brown volumetric flask, and dilute to the scale. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

10.4.4 Vitamin B_6 standard mixed stock solution (50.0 μg/mL): respectively and accurately draw 5.00 mL of pyridoxamine, pyridoxal and pyridoxine standard stock solution (1.00 mg/mL) into a 100 mL brown volumetric flask, and use 0.1 mol/L hydrochloric acid solution to dilute to the scale. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

10.4.5 Vitamin B_6 standard mixed working solution (5.00 μg/mL): accurately draw 1.00 mL of vitamin B_6 standard mixed stock solution (50.0 μg/mL) into a 10 mL brown volumetric flask, use mobile phase A to dilute to the scale. Prepare it right before use.

10.4.6 Vitamin B_6 standard mixed working solution (500 ng/mL): accurately draw 1.00 mL of vitamin B_6 standard mixed working solution (5.00 μg/mL) into a 10 mL brown volumetric flask, use mobile phase A to dilute to the scale. Prepare it right before use.

10.4.7 Vitamin B_6 standard mixed working solution (50.0 ng/mL): accurately draw 1.00 mL of vitamin B_6 standard mixed working solution (500 ng/mL) into a 10 mL brown volumetric flask, use mobile phase A to dilute to the scale. Prepare it right before use.

NOTE: the frozen stock solution shall be thawed at room temperature and evenly mixed before use.

10.5 Preparation of Isotope Internal Standard Solutions

10.5.1 $^{13}C_4$-pyridoxine isotope internal standard stock solution (1.00 mg/mL): weigh-take 12.1 mg (accurate to 0.1 mg) of $^{13}C_4$-pyridoxine hydrochloride, use 0.1 mol/L hydrochloric acid solution to dissolve it, transfer into a 10 mL brown volumetric flask and dilute to the scale. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3 months.

10.5.2 D_3-pyridoxal isotope internal standard stock solution (1.00 mg/mL): weigh-take 10.0 mg (accurate to 0.1 mg) of D_3-pyridoxal hydrochloride, use 0.1 mol/L hydrochloric acid solution to dissolve it, transfer into a 10 mL brown volumetric flask and dilute to the scale. Then, transfer it to a brown glass reagent bottle, and store it in the dark at –20 °C. It shall remain valid for 3

11.9 Constant-temperature water bath.

12 Analytical Procedures

12.1 Specimen Preparation

For powdery samples (milk powder and rice noodles, etc.), evenly mix them and conduct direct sampling. For liquid samples, evenly shake them and reserve them for later use. For flaky and granular samples, use a high-speed pulverizer to grind them into powder or use a homogenizer to homogenize them, and seal for later use. The prepared specimens shall be kept refrigerated in the dark and determined as soon as possible.

12.2 Specimen Treatment

12.2.1 Specimens containing starch

12.2.1.1 Solid specimens: weigh-take 1 g ~ 5 g (accurate to 0.01 g) of evenly mixed solid specimen, add 0.4 mL of vitamin B_6 isotope internal standard mixed working solution, then, add about 25 mL of warm water (45 °C ~ 50 °C) to dissolve it. Use hydrochloric acid solution or sodium hydroxide solution to adjust the pH to 6.0 ~ 6.5, add 0.1 g of α-amylase into a 150 mL stoppered conical flask. Shake and evenly mix it, then, fill the conical flask with nitrogen, plug the flask, and place it in an incubator at 50 °C ~ 60 °C for about 30 min. Take it out and cool to room temperature.

12.2.1.2 Liquid specimens: weigh-take 5 g ~ 20 g (accurate to 0.01 g) of evenly mixed liquid specimen, add 0.4 mL of vitamin B_6 isotope internal standard mixed working solution, then, add 20 mL of water. Use hydrochloric acid solution or sodium hydroxide solution to adjust the pH to 6.0 ~ 6.5, add 0.1 g of α-amylase into a 150 mL stoppered conical flask. Shake and evenly mix it, then, fill the conical flask with nitrogen, plug the flask, and place it in an incubator at 50 °C ~ 60 °C for about 30 min. Take it out and cool to room temperature.

12.2.2 Starch-free specimens

12.2.2.1 Solid specimens: weigh-take 1 g ~ 5 g (accurate to 0.01 g) of evenly mixed solid specimen into a 150 mL stoppered conical flask, accurately add 0.4 mL of vitamin B_6 isotope internal standard mixed working solution, then, add about 25 mL of water to dissolve it, shake and evenly mix it.

12.2.2.2 Liquid specimens: weigh-take 5 g ~ 20 g (accurate to 0.01 g) of evenly mixed liquid specimen into a 150 mL stoppered conical flask, accurately add 0.4 mL of vitamin B_6 isotope internal standard mixed working solution, then, add 20 mL of water, and perform vortex mixing.

12.2.3 Preparation of test solution

Use hydrochloric acid solution to adjust the pH of the above-mentioned specimen solution to 1.7 ± 0.1, and let it stand for about 1 min. Then, use sodium hydroxide solution (0.1 mol/L) to

17.4 Preparation of Standard Solutions

17.4.1 Pyridoxine standard stock solution (1.00 mg/mL): accurately weigh-take 60.8 mg (accurate to 0.1 mg) of pyridoxine hydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it, then, dilute to 50 mL. Store it in the dark in the refrigerator at –20 °C. It shall remain valid for 3 months.

17.4.2 Pyridoxal standard stock solution (1.00 mg/mL): accurately weigh-take 60.9 mg (accurate to 0.1 mg) of pyridoxal hydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it, then dilute to 50 mL. Store it in the dark in the refrigerator at –20 °C. It shall remain valid for 3 months.

17.4.3 Pyridoxamine standard stock solution (1.00 mg/mL): accurately weigh-take 71.7 mg (accurate to 0.1 mg) of pyridoxamine dihydrochloride reference material, use 0.1 mol/L hydrochloric acid solution to dissolve it, then dilute to 50 mL. Store it in the dark in the refrigerator at –20 °C. It shall remain valid for 3 months.

17.4.4 Vitamin B_6 standard mixed working solution (20.0 μg/mL): respectively and accurately draw 1.0 mL of pyridoxine, pyridoxal and pyridoxamine standard stock solutions (1.00 mg/mL). Use 0.1 mol/L hydrochloric acid solution to dilute to 50 mL. Prepare it right before use.

17.4.5 Vitamin B_6 standard mixed working solution (2.00 μg/mL): accurately draw 5.00 mL of pyridoxine, pyridoxal and pyridoxamine standard mixed working solution (20.0 μg/mL), use 0.1 mol/L hydrochloric acid solution to dilute to 50 mL. Prepare it right before use.

17.4.6 Vitamin B_6 standard mixed series working solutions: respectively and accurately draw an appropriate amount of vitamin B_6 standard mixed working solution (2.00 μg/mL) into a 100 mL volumetric flask, use water to dilute them. The concentrations of this standard series are respectively: 0.04 μg/mL, 0.10 μg/mL, 0.20 μg/mL, 0.40 μg/mL, 0.60 μg/mL and 1.00 μg/mL. Prepare them right before use.

NOTE 1: please refer to Appendix A for the concentration correction method of standard stock solution.

NOTE 2: the frozen stock solution shall be thawed at room temperature and evenly mixed before use.

18 Instruments and Equipment

18.1 High-performance liquid chromatograph: equipped with a fluorescence detector.

18.2 Balance: with a division value of 0.01 g and 0.1 mg.

18.3 pH meter: with an accuracy of 0.01.

18.4 Vortex mixer.

18.5 Ultrasonic oscillator.

18.6 Spectrophotometer.

18.7 Constant-temperature incubator, or one with equivalent performance.

19 Analytical Procedures

19.1 Specimen Preparation

19.1.1 Specimens containing starch

19.1.1.1 Solid specimens: weigh-take 1 g ~ 5 g (accurate to 0.01 g) of evenly mixed solid specimen into a 150 mL conical flask, add about 25 mL of 45 °C ~ 50 °C water, and evenly mix it. Add about 0.5 g of α-amylase. After evenly mixing it, fill the conical flask with nitrogen, plug the flask and place it in an incubator at 50 °C ~ 60 °C for about 30 min. Take it out and cool to room temperature.

19.1.1.2 Liquid specimens: weigh-take 5 g ~ 20 g (accurate to 0.01 g) of evenly mixed liquid specimen into a 150 mL conical flask, and evenly mix it. Add about 0.5 g of α-amylase. After evenly mixing it, fill the conical flask with nitrogen, plug the flask and place it in an incubator at 50 °C ~ 60 °C for about 30 min. Take it out and cool to room temperature.

19.1.2 Starch-free specimens

19.1.2.1 Solid specimens: weigh-take 1 g ~ 5 g (accurate to 0.01 g) of evenly mixed solid specimen into a 150 mL conical flask, add about 25 mL of 45 °C ~ 50 °C water, and evenly mix it. Let it stand for 5 min ~ 10 min, then, cool to room temperature.

19.1.2.2 Liquid specimens: weigh-take 5 g ~ 20 g (accurate to 0.01 g) of evenly mixed liquid specimen into a 150 mL conical flask. Let it stand for 5 min ~ 10 min.

19.1.3 Preparation of test solution

Use hydrochloric acid solution to adjust the pH of the above-mentioned specimen solution to 1.7 ± 0.1 and let it stand for about 1 min. Then, use sodium hydroxide solution to adjust the pH of the specimen solution to 4.5 ± 0.1. Put the above-mentioned conical flask into an ultrasonic oscillator and perform ultrasonic oscillation for about 10 min. Transfer the specimen solution to a 50 mL volumetric flask and use water to rinse the conical flask. Combine the washing solutions in a 50 mL volumetric flask and use water to dilute to 50 mL. Take another 50 mL conical flask, put in a funnel and filter paper, pour the diluted specimen solution into it, and naturally filter it. Filter the filtrate through 0.45 μm microporous filter membrane, then, transfer 1 mL to a brown sample injection bottle, and use it as the specimen solution to be tested.

NOTE: during the operation, avoid strong light exposure; when extracting gel candies and jelly specimens, they can be heated and dissolved in 70 °C water bath.

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