GB

NATIONAL STANDARD OF THE

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National food safety standard - Determination of synthetic colorants in food

食品安全国家标准　食品中合成着色剂的测定

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National food safety standard - Determination of synthetic colorants in food

1 Scope

This standard specifies the liquid chromatography method for the determination of synthetic colorants in food.

This standard is applicable to the determination of 11 synthetic colorants (tartrazine, new red, amaranth, indigo, ponceau 4R, sunset yellow, allura red, brilliant blue, acid red, quinoline yellow, and erythrosine) in food.

2 Principle

The synthetic colorant in the sample is extracted with ethanol ammonia solution, purified by solid-phase extraction, then measured with a high-performance liquid chromatograph equipped with a diode array detector, and quantified by the external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade and the water is first-grade water specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH_4O): chromatographically pure.

3.1.2 Methanol (CH_4O).

3.1.3 Petroleum ether: The boiling range is 30 ℃~60 ℃.

3.1.4 Absolute ethanol (C_2H_6O).

3.1.5 Ammonia ($NH_3 \cdot H_2O$): The content is 20%~25%.

3.1.6 Ammonium acetate ($C_2H_7O_2N$): chromatographically pure.

3.1.7 Formic acid (CH_2O_2): The content is 98%.

3.2 Reagent preparation

3.2.1 Ethanol ammonia solution: Measure 700 mL of absolute ethanol, add 4 mL of ammonia, dilute to 1 L with water, and mix well.

3.2.2 5% methanol aqueous solution: Pipette 5 mL of methanol (3.1.2), dilute with water and make up to 100 mL, and mix well.

3.2.3 2% ammonia methanol solution: Pipette 2 mL of ammonia and dilute to 100 mL with methanol (3.1.2).

3.2.4 Ammonium acetate solution (20 mmol/L): Weigh 1.54 g of ammonium acetate, dissolve it in water, and dilute it to 1000 mL.

3.2.5 Ammonium acetate buffer solution, pH=9.0: Add ammonia water to the ammonium acetate solution (3.2.4) and adjust the pH to 9.0.

3.2.6 2% formic acid aqueous solution: Pipette 2 mL of formic acid and dilute to 100 mL with water.

3.3 Standards

3.3.1 Tartrazine (CAS No.: 1934-21-0): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.2 New red (CAS No.: 220658-76-4): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.3 Amaranth (CAS No.: 915-67-3): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.4 Indigo (CAS No.: 860-22-0): with a purity of ≥90.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.5 Carmine (CAS No.: 2611-82-7): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.6 Sunset yellow (CAS No.: 2783-94-0): with a purity of ≥90.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.7 Allura red (CAS No.: 25956-17-6): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.8 Brilliant blue (CAS No.: 3844-45-9): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.9 Acid red (CAS No.: 3567-69-9): with a purity of ≥90.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.10 Quinoline yellow (CAS No.: 8004-92-0): with a purity of ≥95.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.3.11 Erythrosine (CAS No.: 16423-68-0): with a purity of ≥90.0%, or a standard product obtained national certification and awarded a reference material certificate.

3.4 Preparation of standard solution

3.4.1 Standard stock solution (1.0 mg/mL): Accurately weigh 100 mg (accurate to 0.1 mg) of each of tartrazine, new red, amaranth, ponceau 4R, sunset yellow, allura red, brilliant blue, acid red, quinoline yellow and erythrosine, converted to 100 % by mass according to their purity, dissolve in water and place in 100 mL volumetric flasks respectively, dilute to volume, and shake well to obtain standard stock solutions with a concentration of 1.0 mg/mL. The standard stock solution can be stored at 4 °C and away from light for 6 months. Prepare the indigo standard solution fresh before use.

3.4.2 Mixed standard intermediate solution (50.0 μg/mL): Take 5.00 mL of each of the above standard stock solution (3.4.1) and indigo standard solution (1.0 mg/mL) into a 100 mL volumetric flask, dilute to the mark with water, and shake well to obtain the mixed standard intermediate solution (the concentration of each synthetic colorant is 50.0 μg/mL). Prepare the solution fresh before use.

3.4.3 Standard series working solutions: Take 0.2 mL, 0.5 ml, 1.0 mL, 2.0 mL, 5.0 mL, and 10.0 mL of the mixed standard intermediate solution (3.4.2) in 50 mL volumetric flasks, dilute to the mark with water, and shake well to obtain standard series working solutions. The concentrations are 0.2 μg/mL, 0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL, 5.0 μg/mL and 10.0 μg/mL, respectively.

4 Instruments and equipment

4.1 High-performance liquid chromatograph: with a diode array detector.

4.2 Balance: The sensitivities are 1 mg and 0.1 mg, respectively.

4.3 pH meter: The accuracy is 0.01.

4.4 Electric stirrer: The speed range is 30 r/min~2000 r/min.

4.5 Vortex mixer.

4.6 Ultrasonic generator or constant temperature shaker: The ultrasonic power is not less than 700 W; the temperature control range is 20 ℃~80 ℃; the shaker speed range is 10 r/min~500 r/min.

4.7 High-speed centrifuge: The speed is not less than 15000 r/min.

(3.2.1), vortex for 1 min, extract for 20 min at 50 ℃ by ultrasonic or shaking (with a speed of ≥250 r/min), and centrifuge at 8000 r/min for 5 min; take the supernatant and place it in a 50 mL volumetric flask, add about 5 mL~10 mL of ethanol ammonia solution (3.2.1) each time, repeat the extraction operation until the supernatant has no obvious color, combine the supernatants after centrifugation, and make up to 50 mL with ethanol ammonia solution (3.2.1) to obtain the extract. Accurately pipette 10 mL of the extract, concentrate it to about 3 mL with nitrogen at 50 ℃, add a total of 10 mL of 5% methanol aqueous solution 2~3 times to dissolve it, and use the solution as the liquid to be purified.

5.1.2.3 Samples with high oil content (cocoa products, chocolate and chocolate products, formulated milk powder, modulation cream powder, formulated condensed milk, puffed food, processed nuts and seeds, cooked beans, pastries, cooked meat products, compound seasonings, ice cream, popsicles, etc.)

Accurately weigh 2 g of the sample (accurate to 0.001 g), place it in a 50 mL stoppered centrifuge tube, add 20 mL of petroleum ether, vortex for 1 min, extract by ultrasonic or shaking (with a speed of ≥250 r/min) for 10 min, centrifuge at 8000 r/min for 5 min, and discard the supernatant; repeat the extraction once for samples with high oil content, and discard the supernatant; add 25 mL of ethanol ammonia solution (3.2.1), vortex for 1 min, extract by ultrasonic or shaking (with a speed of ≥250 r/min) at 50 ℃ for 20 min, and centrifuge at 8000 r/min for 5 min (if the extract is still turbid after centrifugation, transfer it to a special tube for high-speed centrifuge and centrifuge at 15000 r/min for 5 min); take the supernatant and place it in a 50 mL volumetric flask, and operate the steps "add about 5 mL~10 mL of ethanol ammonia solution each time, ... as the liquid to be purified" as described in 5.1.2.2.

5.1.3 Sample purification

5.1.3.1 Activation

Activate the solid phase extraction column (4.10) with 6 mL of methanol (3.1.2) and 6 of mL water in sequence, and keep the column moist.

5.1.3.2 Sample loading

Immediately after activation, load the liquid to be purified obtained in 5.1.2 onto the solid-phase extraction column at a flow rate of 2~3 seconds per drop.

5.1.3.3 Rinsing

Rinse the solid phase extraction column with 6 mL of 2% formic acid aqueous solution and 6 mL of methanol (3.1.2) in sequence, discard the washing liquid, and use the vacuum to pump humidity out for 2 minutes until the column is nearly dry.

5.1.3.4 Elution

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