



**NATIONAL STANDARD OF THE  
PEOPLE'S REPUBLIC OF CHINA**

**GB 5009.35-2023**

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**National food safety standard - Determination of synthetic  
colour in foods**

食品安全国家标准 食品中合成着色剂的测定

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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 5009.35-2023 covers the liquid chromatographic determination of synthetic colours in food, for the eleven colorants named in its scope, tartrazine among them. The colorant is extracted from the sample with an ethanol-ammonia solution, cleaned up by solid-phase extraction, then measured on a high-performance liquid chromatograph fitted with a diode array detector and quantified against external standards. Around that sequence the standard sets the reagents and materials, the instruments and equipment, the analysis steps, the presentation of results, the precision and the further method information gathered in its closing clause; Appendix A carries the chromatogram of the standard solution, so an analyst can check that peaks are being assigned to the right dye. Synthetic colours are permitted additives, but only in named foods and within set limits, and colour is one of the easiest things to add for commercial reasons and one of the hardest to see afterwards. Enforcing those permissions rests on a method that says which dye is present and how much of it, reproducibly enough that two laboratories reach the same verdict on one sample. A mandatory national food safety standard, used by inspection laboratories, regulators and manufacturers checking their own additive declarations.

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 ( $\text{CH}_4\text{O}$ ): chromatographically pure.

3.1.2 Methanol ( $\text{CH}_4\text{O}$ ).

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

3.1.4 Absolute ethanol ( $\text{C}_2\text{H}_6\text{O}$ ).

3.1.5 Ammonia ( $\text{NH}_3 - \text{H}_2\text{O}$ ): The content is 20%~25%.

3.1.6 Ammonium acetate ( $\text{C}_2\text{H}_7\text{O}_2\text{N}$ ): chromatographically pure.

3.1.7 Formic acid ( $\text{CH}_2\text{O}_2$ ): 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  $\geq 95.0\%$ , 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  $\geq 95.0\%$ , 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  $\geq 95.0\%$ , 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  $\geq 90.0\%$ , 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  $\geq 95.0\%$ , 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  $\geq 90.0\%$ , 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  $\geq 95.0\%$ , 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  $\geq 95.0\%$ , 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  $\geq 90.0\%$ , 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  $\geq 95.0\%$ , 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  $\geq 90.0\%$ , 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 ug/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 ug/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 ug/mL, 0.5 ug/mL, 1.0 ug/mL, 2.0 ug/mL, 5.0 ug/mL and 10.0 ug/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 degrees C~80 degrees C; the shaker speed range is 10 r/min~500 r/min.