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# Method of Test for Colorants in Foods (2)

## 1. Scope

This method is applicable to the determination of 16 colorants (tartrazine, etc. listed in the attached table) in beverage, candy and jelly candy (besides the products containing corn syrup).

## 2. Method

After extraction, colorants are determined by high performance liquid chromatograph (HPLC).

**2.1.** Equipment

**2.1.1.** High performance liquid chromatograph.

**2.1.1.1.** Detector: photodiode array detector.

**2.1.1.2.** Column: Acquity UPLC® BEH C18, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

**2.1.2.** Centrifuge: centrifugal force ≥ 15000 ×g.

**2.1.3.** Ultrasonicator: temperature control ≥ 38°C.

**2.1.4.** Tissue homogenizer: rotary speed ≥ 1000 rpm, or an equivalent product.

**2.1.5.** Vortex mixer.

**2.2.** Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
n-Hexane, HPLC grade;
Glacial acetic acid, reagent grade;
Ammonium solution (25%), reagent grade;
Ammonium acetate, trace grade;
Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);
Tartrazine and other colorants listed in the attached table, reference standard.

**2.3.** Apparatus

**2.3.1.** Volumetric flask: 50 mL and 500 mL.

**2.3.2.** Centrifuge tube: 15 mL and 50 mL, PP.

**2.3.3.** Ceramic homogenizer(note): Bond Elut QuEChERS P/N 5982-9313, or an equivalent product.

Note: Ceramic homogenizer can be used depending on the

texture of the sample.

**2.3.4.** Membrane filter: 0.45 μm, PVDF.

**2.4.** Reagents preparation

**2.4.1.** 2.5% ammonium solution: dilute 5 mL of ammonium solution with deionized water to 50 mL.

**2.4.2.** 10% acetic acid solution: dilute 5 mL of acetic acid with deionized water to 50 mL.

**2.4.3.** 0.125% ammonium solution: dilute 5 mL of ammonium solution with deionized water to 1000 mL.

**2.5.** Mobile phase

**2.5.1.** Solvent A: dissolve 7.71 g of ammonium acetate with 100 mL of deionized water, add 25 mL of acetonitrile and dilute with deionized water to 1000 mL. Adjust pH to 7.8 with 2.5% ammonium solution and filter with a membrane filter.

**2.5.2.** Solvent B: dissolve 7.71 g of ammonium acetate with 100 mL of deionized water, add 500 mL of acetonitrile and dilute with deionized water to 1000 mL. Adjust pH to 7.8 with 2.5% ammonium solution and filter with a membrane filter.

**2.6.** Standard solution preparation

Accurately weigh about 100 mg of colorant reference standards into a 10 mL volumetric flask separately, dissolve and dilute with deionized water to volume as the standard stock solutions. Store at 4°C in dark. When to use, mix appropriate volume of each standard stock solution and dilute with mobile phase solvent A to 0.1 – 5 μg/mL as the standard solutions.

**2.7.** Sample solution preparation

**2.7.1.** Beverage

Carbonated sample should be ultrasonicate for 10 min to remove carbon dioxide. Accurately weigh about 5 g of well-mixed sample, add 30 mL of deionized water. Adjust pH to 7.3 – 8.3 with 2.5% ammonium solution or 10% acetic acid solution, dilute to 50 mL with deionized water. Centrifuge at 15000 ×g for 10 min, collect the supernatant as the sample

solution.

**2.7.2.** Jelly candy and candy

Transfer about 5 g of the fine-cut jelly candy or homogenized candy accurately weighed into a 50 mL centrifuge tube. Add 10 mL of 0.125% ammonium solution and two ceramic homogenizers. Shake at 1000 rpm by tissue homogenizer for 1 min or vortex-mix for 1 min. Ultrasonicate at 38°C in a water bath for 30 min, centrifuge at 5000 ×g for 5 min and collect the supernatant. Add 10 mL of 0.125% ammonium solution to the residue and repeat extraction procedure once. Combine the supernatant, adjust pH to 7.3 – 8.3 with 2.5% ammonium solution or glacial acetic acid, and dilute to 50 mL with deionized water. Transfer 2.5 mL of the solution into a 15 mL centrifuge tube, add 1 mL of hexane. Vortex for 1 min and centrifuge at 5000 ×g for 10 min. Take 1 mL of the lower layer and centrifuge at 15000 ×g for 10 min. Take the supernatant as the sample solution.

**2.8.** Identification and quantification

Accurately inject 10 μL of the sample solution and the standard solutions into HPLC separately, and operate according to the following conditions. Identify each colorant based on the retention time and the absorption spectrum. Calculate the amount of each colorant in the sample using the following formula:

The amount of each colorant in the sample (mg/kg) = $\frac{C \times V}{M}$

Where,

C: the concentration of each colorant in the sample solution calculated by the standard curve (μg/mL)

V: the final make-up volume of the sample (50 mL)

M: the weight of the sample (g)

HPLC operating conditions (note):

Photodiode array detector: the quantitative wavelength listed in the attached table.

Column: Acquity UPLC® BEH C18, 1.7 μm, 2.1 mm i.d. x 10 cm.

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Column temperature: 50°C.

Mobile phase solution: a gradient program of solvent A and solvent B is as follows.

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0 → 2 | 100 → 90 | 0 → 10 |
| 2 → 7 | 90 → 90 | 10 → 10 |
| 7 → 11 | 90 → 54 | 10 → 46 |
| 11 → 13 | 54 → 54 | 46 → 46 |
| 13 → 14 | 54 → 53 | 46 → 47 |
| 14 → 16 | 53 → 53 | 47 → 47 |
| 16 → 22 | 53 → 10 | 47 → 90 |
| 22 → 25 | 10 → 10 | 90 → 90 |
| 25 → 25.1 | 10 → 100 | 90 → 0 |
| 25.1 → 30 | 100 → 100 | 0 → 0 |

Flow rate: 0.4 mL/min.

Injection volume: 10 μL.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

**Remark**

1. Limit of quantification (LOQ) for each colorant is 1 mg/kg.
2. Further validation should be performed when interference compounds appear in samples.

**References**

1. Harp, B. P., Miranda-Bermudez, E. and Barrows, J. N. 2013. Determination of seven certified color additives in food products using liquid chromatography. J. Agric. Food Chem. 61: 3726-3736.
2. Martin, F., Oberson, J., Meschiari, M. and Munari, C. 2016. Determination of 18 water-soluble artificial dyes by LC–MS in selected matrices. Food Chem. 197: 1249-1255.

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Table. Quantitative wavelength of 16 colorants.

| NO. | Analyte | CAS # | Quantitative wavelength (nm) |
|---|---|---|---|
| 1 | Tartrazine | 1934-21-0 | 427 |
| 2 | Amaranth | 915-67-3 | 500 |
| 3 | Indigo Carmine | 860-22-0 | 620 |
| 4 | New Coccin | 2611-82-7 | 500 |
| 5 | Sunset Yellow FCF | 2783-94-0 | 500 |
| 6 | Brilliant Black BN | 2519-30-4 | 550 |
| 7 | Allura Red AC | 25956-17-6 | 500 |
| 8 | Azorubine | 3567-69-9 | 500 |
| 9 | Green S | 3087-16-9 | 620 |
| 10 | Fast Green FCF | 2353-45-9 | 620 |
| 11 | Brilliant Blue FCF | 3844-45-9 | 620 |
| 12 | Erythrosine | 16423-68-0 | 500 |
| 13 | Patent Blue V | 20262-76-4 | 620 |
| 14 | Sulforhodamine B | 3520-42-1 | 550 |
| 15 | Phloxine B | 18472-87-2 | 550 |
| 16 | Rose Bengal | 632-69-9 | 550 |

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## Reference chromatogram

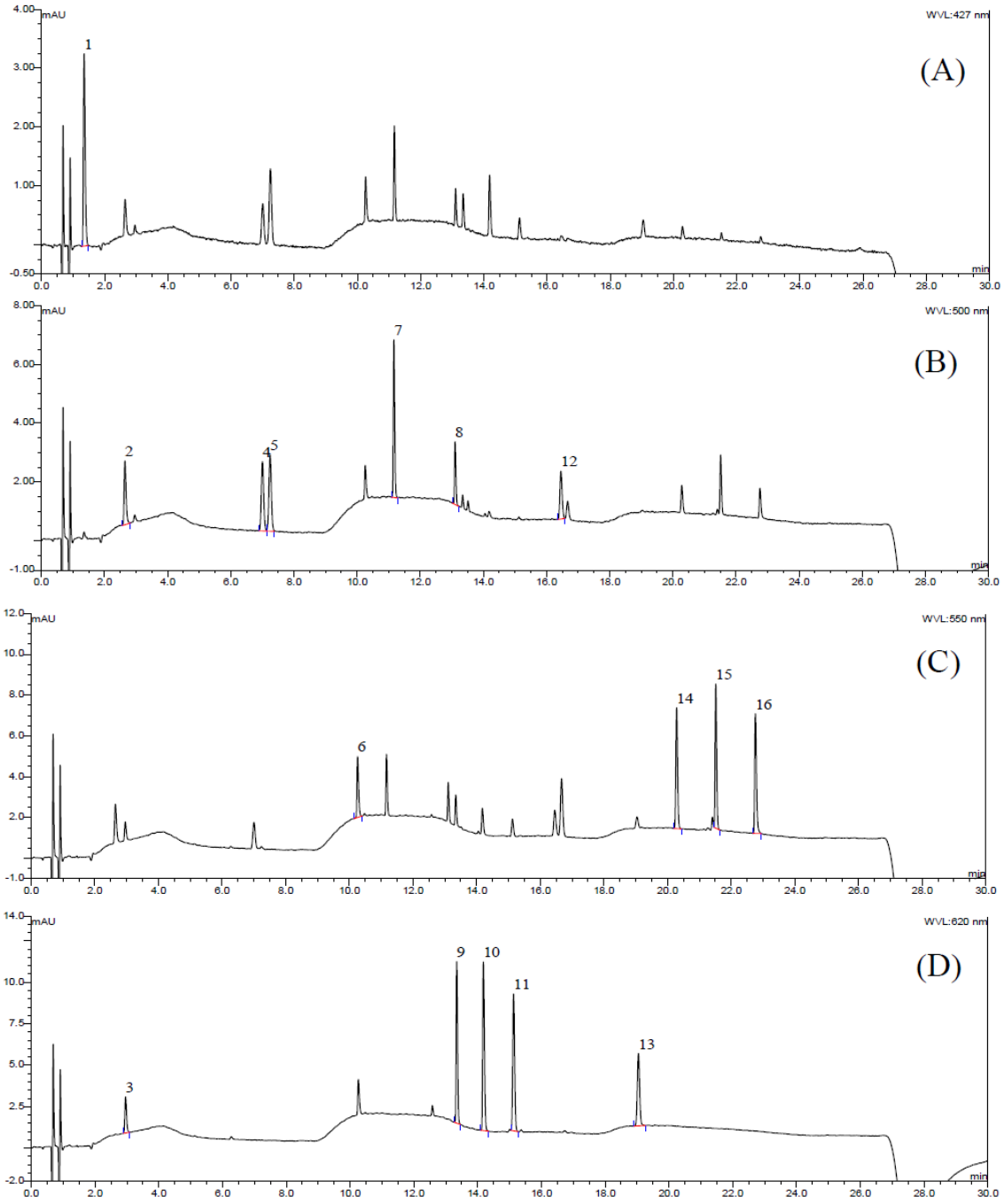


Figure. HPLC chromatograms of 16 colorant standards at wavelength of 427 nm (A), 500 nm (B), 550 nm (C) and 620 nm (D).

1. Tartrazine; 2. Amaranth; 3. Indigo Carmine; 4. New Coccin; 5. Sunset Yellow FCF; 6. Brilliant Black BN; 7. Allura Red AC; 8. Azorubine; 9. Green S; 10. Fast Green FCF; 11. Brilliant Blue FCF; 12. Erythrosine; 13. Patent Blue V; 14. Sulforhodamine B; 15. Phloxine; 16. Rose Bengal