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National food safety standard -

Determination of allura red in foods

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National food safety standard - Determination of allura red in foods

1 Scope

This standard specifies the determination method of allura red in soft drinks, hard candy, cakes and ice cream.

This standard applies to the determination of allura red in soft drinks, hard candy, cakes and ice cream.

2 Principle

The allura red is adsorbed by the polyamide powder under acidic conditions, and desorbed under alkaline conditions, then it is separated by paper chromatography, and qualitatively and quantitatively compared with the standard.

3 Reagents and materials

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

3.1 Reagents

3.1.1 Methanol (CH_3OH).

3.1.2 petroleum ether: boiling range 30 °C ~ 60 °C.

3.1.3 Sulfuric acid (H_2SO_4): excellent grade pure.

3.1.4 Ethanol (CH_3CH_2OH).

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

3.1.6 Citric acid ($C_6H_8O_7 \cdot H_2O$).

3.1.7 Sodium tungstate ($Na_2WO_4 \cdot H_2O$).

3.1.8 Butanone (C_4H_8O).

3.1.9 Sodium citrate ($C_6H_5Na_3O_7$).

converted in accordance with the actual purity of allura red), USE water to dissolve and make its volume reach to 25 mL, the allura red concentration is 1.0 mg/mL.

3.4.2 Allura red standard use solution (0.1 mg/mL): PIPETTE 5.0 mL of allura red standard stock solution in a 50 mL volumetric flask, ADD water to dilute it to 50 mL.

4 Instruments and equipment

4.1 Visible spectrophotometer.

4.2 Electronic balance: The sensitivity is 0.001 g and 0.0001 g.

4.3 Microinjector: 10 μL, 50 μL.

4.4 Developer tank

4.5 Electric blower.

4.6 Centrifuge.

4.7 Constant temperature water bath.

5 Analytical procedures

5.1 Specimen preparation

5.1.1 Soft water: After heating the sample to remove carbon dioxide, WEIGH 10 g (accurate to 0.001 g) of sample in a beaker, then USE 20% citric acid to adjust the pH to acidity, ADD 0.5 g ~ 1.0 g of polyamide powder to absorb the pigment, TRANSFER polyamide powder adsorbing the pigment into a funnel for filtration, USE acidic hot water at pH4 to rinse it for many times (about 200 mL), to wash away sugar and the like. If there is a natural pigment, USE methanol-formic acid solution to rinse it for 1 ~ 3 times, 20 mL for each rinsing, until the rinsing solution is colorless. Then USE water at 70 °C to make multiple rinsing until the flowing out solution is neutral. In the rinsing process, it shall fully mix it and then use ethanol-ammonia solution to desorb the pigment for many times, COLLECT all the desorption solution, REMOVE the ammonia on a water bath, EVAPORATE it to about 2 mL, TRANSFER it to a 5 mL volumetric flask, USE 50% ethanol to rinse the evaporating dish for many times. CONTAIN the rinsing solution into a 5 mL volumetric flask, USE 50% ethanol to make its volume to the mark. This solution is retained for paper chromatography.

5.1.2 Hard candy: WEIGH 10 g (accurate to 0.001g) of the pulverized sample, ADD 30 mL of water, HEAT to dissolve it. If the pH of the sample solution is

to precipitate the protein, LET it be standing for 2 min, then USE ethanol-ammonia to adjust pH to alkaline. TRANSFER the solution into a centrifuge tube, CENTRIFUGE it at 5000 r/min for 15 min, POUR out the supernatant, EVAPORATE off ethanol in water bath, then USE the citric acid solution to adjust pH to acidity, ADD 0.5 g ~ 1.0 g of polyamide powder to absorb the pigment, TRANSFER polyamide powder adsorbing the pigment into a funnel for filtration, USE acidic hot water at pH4 to rinse it for many times (about 200 mL), to wash away sugar and the like. If there is a natural pigment, USE methanol-formic acid solution to rinse it for 1 ~ 3 times, 20 mL for each rinsing, until the rinsing solution is colorless. Then USE water at 70 °C to make multiple rinsing until the flowing out solution is neutral. In the rinsing process, it shall fully mix it and then use ethanol-ammonia solution to desorb the pigment for many times, COLLECT all the desorption solution, REMOVE the ammonia on a water bath, EVAPORATE it to about 2 mL, TRANSFER it to a 5 mL volumetric flask, USE 50% ethanol to rinse the evaporating dish for many times. CONTAIN the rinsing solution into a 5 mL volumetric flask, USE 50% ethanol to make its volume to the mark. This solution is retained for paper chromatography.

5.2 Qualitative

TAKE the chromatographic paper, ADD 3 μL ~ 10 μL of sample treatment solution and 1 μL of allura red standard use solution on the 2 cm starting line from the bottom edge, respectively, HANG it on the developer tank containing the developer 1, the developer 2, and the developer 3. USE the up-rising method to extend it, when the front edge of the reagent reaches to the position of 15 cm, TAKE the filter paper out and DRY it naturally in air, COMPARE it with the standard spot for qualitative.

5.3 Quantification

5.3.1 Preparation of standard curve

PIPETTE 0.0 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL allura red standard use solution, respectively, PLACE it in a 10 mL colorimetric tube respectively, ADD water to each tube to dilute it to the mark, the concentration is respectively 0 μg/mL, 2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL, and 10 μg/mL. USE a 1 mL cuvette, USE a zero tube to adjust the zero point, at the wavelength of 500 nm, DETERMINE the absorbance and DRAW the standard curve.

5.3.2 Determination of samples

TAKE the chromatographic paper and ADD 0.20 mL of sample treatment solution on the starting line 2 cm from the bottom edge, in the shape of a strip from left to right. ADD 1 μL of allura red standard solution at the right side of the paper, UNFLOD it as required, TAKE it out and DRY it naturally. CUT off the color strip of the sample, USE a small amount of hot water to rinse it for many

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