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Beet Red

Definition Beet Red is obtained from beet roots, of which coloring principles are isobetanine and betanine. Commercial products may contain dextrin or lactose.

Color Value The Color Value ($E_{1\text{cm}}^{10\%}$) of Beet Red is not less than 15 and is in a range of 90 - 110% of the labeled value.

Description Beet Red is red-purple to dark purple powder, lumps, paste, or liquid having a slightly characteristic odor.

Identification (1) Weigh the equivalent of 1 g of Beet Red calculated in terms of Color Value 15 from the labeled value and dissolve in 50 ml of acetate buffer (pH 5.4). A red-purple color develops.

(2) To 5 ml of the above solution (1), add 1 ml of sodium hydroxide solution. The color of the solution changes to yellow.

(3) Beet red in acetate buffer (pH 5.4) exhibits an absorption maximum at a wavelength of 525 - 535nm.

(4) Weigh the equivalent of 1 g of Beet Red calculated in terms of color value 15 from the labeled value, dissolve in 5 ml of water, add 20 ml of methanol, and mix. Centrifuge the solution at 3,000 rpm for 10 minutes, and use the supernatant as the test solution. Measure 8 μl of the test solution and perform Thin-Layer Chromatography without a control solution, using a *n*-butanol - water - acetic acid mixture (4 : 3 : 2) as the developing solvent. A purple spot is observed at R_f around 0.3 - 0.5. For the thin-layer plate, use a plate prepared by applying microcrystalline cellulose for thin-layer chromatography dried at 60 - 80 °C for 20 minutes for the support. Stop the development when the solvent front rises about 10 cm above the original line, air-dry, and observe.

Purity (1) Heavy metals Not more than 40 $\mu\text{g/g}$ as Pb (0.50 g, Method 2, Control solution Lead standard solution 2.0 ml)

(2) Lead Not more than 10 $\mu\text{g/g}$ as Pb (1.0 g, Method 1)

(3) Arsenic Not more than 4.0 $\mu\text{g/g}$ as As_2O_3 (0.50 g, Method 3, Apparatus B)

(4) Nitrate Not more than 0.27 % as NO_3 per Color Value 15

Test Solution Weigh accurately about 0.1 g of Beet Red, dissolve in exactly 100 ml of water.

Standard Solution Measure exactly 0.2 ml, 1 ml, 10 ml, and 50 ml of Nitrate Ion Standard Stock Solution, add water to each, and make 100 ml.

On 20 μl each of the test solution, the standard solutions, and the Standard Stock Solution, perform Ion Chromatography. Measure the peak height or peak area of

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nitrate ion for each of the standard solutions and the Standard Stock Solution, and prepare a calibration curve. Measure the peak height or peak area of nitrate ion for the test solution, and obtain the amount using the calibration curve.

Operating conditions

Detector: Conductometer.

Column packing material: Wholly porous anion exchange resin.

Column tube: Stainless steel tube 4.6 - 6.0 mm in internal diameter and 5 - 10 cm in length.

Guard column: Column with the same internal diameter and the same packing material as the column tube.

Eluant: Aqueous solution (pH 4.0) of 2.5 mol/l phthalic acid and 2.4 mmol/l tris(hydroxymethyl)aminomethane.

Temperature: 40 .

Flow rate: 1.5 ml/min.

Color Value Test Proceed as directed in the Color Value Test under the conditions below.

Operating Conditions

Solvent: Acetate buffer (pH 5.4).

Wavelength: Absorption maximum at a wavelength of 525 - 535 nm.