

## D. MONOGRAPHS

### Annatto, Water-soluble

**Definition** Water-soluble Annatto is prepared from the red pericarp of the seed of the annatto tree (*Bixa orellana* L. (*Bixaceae*)) by hydrolysis. The coloring principle is the potassium or sodium salt of norbixin.

**Content** Water-soluble Annatto contains the equivalent of 100 - 125% of the labeled amount of norbixin ( $C_{24}H_{28}O_4=380.48$ ).

**Description** Water-soluble Annatto occurs as red-brown to brown powder, lumps, liquid, or pasty substances, having a slight characteristic odor.

**Identification** (1) Dissolve 0.5 g of Water-soluble Annatto in 20 ml of water, add 2 ml of diluted sulfuric acid (1 : 20), shake, and filter. Wash the residue on the filter paper three times with each 20 ml of water.

(i) Dissolve a portion of the residue in diluted sodium hydroxide solution (1 : 2,500). The solution exhibits absorption maxima at wavelengths of around 452 - 456 nm and 480 - 484 nm.

(ii) Dissolve a portion of the residue in 10 ml of ethanol. Spot one drop of the solution on a filter paper, and air-dry. Drip one drop of antimony trichloride TS. The yellow color on the filter paper changes to blue.

(2) To 1 g of Water-soluble Annatto, add 50 ml of water, shake, and filter. Add 2 ml of diluted hydrochloric acid (1 : 4) to the filtrate. A red-brown to yellow-brown precipitate is formed.

**Purity** (1) Free alkali Dissolve 10 g of Water-soluble Annatto in 100 ml of water, shake, add 8 ml of 1 mol/l hydrochloric acid, stir thoroughly, and allow to stand for 30 minutes. The pH of the filtrate is not more than 7.0.

(2) Heavy metals Not more than 10  $\mu\text{g/g}$  as Pb.

**Test Solution** Weigh 2.0 g of Water-soluble Annatto, evaporate to dryness, if necessary, on a water bath.

**Control Solution** Use 2.0 ml of Lead Standard Solution.

**Procedure** Method 2.

(3) Arsenic Not more than 4.0  $\mu\text{g/g}$  as  $\text{As}_2\text{O}_3$  (Coloring Matter Tests).

(4) Absorbance ratio Proceed as directed in (i) of the Identification (1).

When the absorbances of the absorption maxima at 480 - 484 nm and 452 - 456 nm are  $A_1$  and  $A_2$ , respectively,

$A_2/A_1=1.11 - 1.25$ .

**Assay** Weigh accurately 0.1 to 1 g of Water-soluble Annatto, add 0.01 mol/l sodium hydroxide solution to make exactly 100 ml, and mix thoroughly. Measure exactly 1 ml of the solution, and add 0.01 mol/l sodium hydroxide solution to make

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exactly 100 ml. Measure absorbance A of this solution at the maximum absorbancy of a wavelength of around 454 nm, and calculate the content of norbixin by the formula

Content of norbixin ( $C_{24}H_{28}O_4$ )

$$= \frac{A}{3,473} \times \frac{100,000}{\text{Weight (mg) of the sample}} \times 100 (\%)$$