

## ANNATTO EXTRACTS (SOLVENT-EXTRACTED BIXIN)

Prepared at the 80<sup>th</sup> JECFA and published in FAO JECFA Monographs 17 (2015) superseding specifications prepared at the 67<sup>th</sup> JECFA (2006) published in FAO JECFA Monographs 3 (2006). An ADI for bixin of 0 – 12 mg/kg bw was established at the 67<sup>th</sup> JECFA (2006).

### SYNONYMS

Annatto B, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(i)

### DEFINITION

Solvent-extracted bixin is obtained by the removal of the outer coating of the seeds of the annatto tree (*Bixa orellana* L) with one or more of the following food grade solvents: acetone, methanol, hexane, ethanol, isopropyl alcohol, ethyl acetate, alkaline alcohol or supercritical carbon dioxide. The resulting preparation may be acidified, followed by the removal of the solvent, drying and milling.

Solvent-extracted bixin contains several coloured components; the major colouring principle is *cis*-bixin, a minor colouring principle is *trans*-bixin; thermal degradation products of bixin may also be present as a result of processing.

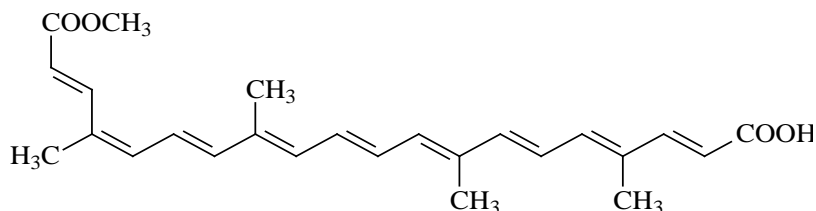
Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name *cis*-Bixin: Methyl (9-*cis*)-hydrogen-6,6'-diapo- $\Psi$ , $\Psi$ -carotenedioate

C.A.S. number *cis*-Bixin: 6983-79-5

Chemical formula *cis*-Bixin: C<sub>25</sub>H<sub>30</sub>O<sub>4</sub>

Structural formula



*cis*-Bixin

Formula weight 394.5

Assay Not less than 85 % colouring matter (expressed as bixin)

### DESCRIPTION

Dark red-brown to red-purple powder

### FUNCTIONAL USES

Colour

### CHARACTERISTICS

#### IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, slightly soluble in ethanol

UV/VIS absorption (Vol. 4) The sample in acetone shows absorbance maxima at about 425, 457 and 487 nm

Thin Layer  
Chromatography

Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250  $\mu\text{m}$ , size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10  $\mu\text{l}$  to the plate. Allow to dry and develop using a mixture of n-butanol, methyl ethyl ketone and 10% aqueous ammonia (3:2:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Bixin and norbixin appear as yellow spots with  $R_f$  values of about 0.50 to 0.45, respectively. Spray with 5% sodium nitrite solution and then with 0.5 mol/l sulphuric acid and the spots immediately decolourise.

PURITY

Norbixin (Vol. 4)

Not more than 2.5 % of total colouring matters.

Residual Solvents

Acetone:	Not more than 30 mg/kg
Methanol:	Not more than 50 mg/kg
Hexane:	Not more than 25 mg/kg
Ethanol:	} Not more than 50 mg/kg, singly or in combination
Isopropyl alcohol:	
Ethyl acetate:	

See description under TESTS

Arsenic (Vol. 4)

Not more than 3 mg/kg  
Determine using an AAS (Hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Lead (Vol. 4)

Not more than 2 mg/kg  
Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Mercury (Vol. 4)

Not more than 1 mg/kg  
Determine using AAS (Cold vapour generation technique). The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

**METHOD OF ASSAY**

Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 2, using 10 ml tetrahydrofuran to dissolve the sample and acetone in place of cyclohexane. Measure the absorbance at the  $A_{\text{max}}$  of about 487 nm. The specific absorbance ( $A_{1\text{cm}}^{1\%}$ ) is 3090.

**TESTS**

Residual solvents

Proceed as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

Stock standard solutions

Add 10 ml dimethylformamide to a 20 ml volumetric flask. Accurately weigh, to within 0.01 mg, each flask. Pipet 250  $\mu\text{l}$  each of chromatography grade methanol, ethanol, isopropanol, and ethyl acetate, and 150  $\mu\text{l}$  each

of acetone and hexane into each of the flask. Reweigh accurately and then fill the flask with dimethylformamide. Mix well.

Standard mixture solution A: Pipet each 3.0 ml of stock standard solution into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Standard mixture solution B: Pipet 4.0 ml solution A into a 10 ml volumetric flask and fill the flask with dimethylformamide.

Standard mixture solution C: Pipet 2.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Standard mixture solution D: Pipet 1.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

#### Samples

Weigh accurately 0.2 g sample into a 20 ml injection vial. Add 2.5 ml dimethylformamide and seal.

#### Standard solutions

Introduce 0.1 ml of the each standard mixture solution (A, B, C and D) into each 20 ml injection vial. Add 2.4 ml dimethylformamide and seal.

#### Standard curves

Place the four standard solutions in the sample tray on head-space gas chromatography. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical condition as described above. Measure the peak area for each solvent. Construct the standard curves by plotting the ratios of the peak areas of each solvent against the concentrations of each solvent (mg/ml) in the standards solutions.

#### Procedure

Place the sample solution in the sample tray on head-space gas chromatograph. Heat vials at 60° for 20 min with continuous agitation.

Analyze using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4.

Measure the peak area for each solvent and obtain the concentration of each solvent (C, mg/ml) from the standard curves.

#### Calculation

Calculate the concentration of each residual solvent in samples from;

$$\text{Residual solvent (mg/kg)} = C \times 2.5/W \times 1000$$

where:

W is weight of sample (g).