

# ANNATTO EXTRACT (SOLVENT-EXTRACTED BIXIN) (TENTATIVE)

Prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). The previous specifications for annatto extracts (solvent-extracted) prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) have been replaced by these and separate specifications for "Annatto extract (solvent-extracted norbixin)". A temporary ADI of 0 – 7 mg/kg bw was established at the 61<sup>st</sup> JECFA (2003).

Information required on chemical characterisation of the non-pigment component of commercial products; an appropriate method to determine norbixin at the specified limit

## SYNONYMS

L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b

## DEFINITION

Seeds from the annatto tree (*Bixa orellana* L.) are extracted with solvent to dissolve pigment. The extract is filtered to remove insoluble material. Subsequent processing involves removal of fats and waxes, solvent removal, crystallisation and drying. Solvents used can be one or more of: hexane, acetone, ethanol, (alkaline) methanol, isopropanol, ethyl acetate. Thermal degradation products may also be present as a result of processing. The major colouring principal is *cis*-bixin. A minor colouring principal is *trans*-bixin.

Chemical name

9'-*cis*-6,6'-Diapocaratene-6,6'-dioic acid, monomethyl ester

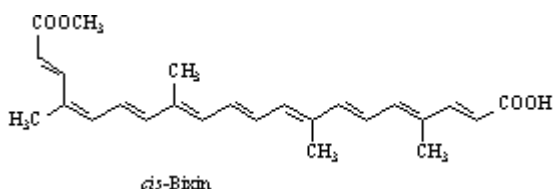
C.A.S. number

*cis*-Bixin 6983-79-5

Chemical formula

C<sub>25</sub>H<sub>30</sub>O<sub>4</sub>

Structural formula



Formula weight

394.51

Assay

Not less than 85% pigment (expressed as bixin)  
Pigment must contain not more than 2.5% norbixin

## 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

The sample in acetone shows absorbance maxima at about 425, 457 and

(Vol. 4) 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 sulfuric acid and the spots immediately decolourise.

#### PURITY

Arsenic (Vol. 4) Not more than 3 mg/kg

Lead (Vol. 4) Not more than 2 mg/kg  
Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in Volume 4 "Instrumental methods".

Mercury (Vol. 4) Not more than 1 mg/kg

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 in Volume 4

**METHOD OF ASSAY** Proceed as directed in Colouring matters, Total Content by Spectrophotometry (Vol. 4), procedure 2 using the following conditions:  
 $w = 0.100 \text{ g}$   
 $V_1 = V_2 = V_3 = 100 \text{ ml}$   
 $v_1 = v_2 = 5 \text{ ml}$   
 $A_{1 \text{ cm}}^{1\%} = 3090$   
 $A_{\text{max}} = \text{about } 487 \text{ nm}$   
Dissolve sample in 10 ml tetrahydrofuran  
Use acetone as solvent