

## ANNATTO EXTRACTS (AQUEOUS-PROCESSED BIXIN)

Prepared at the 67<sup>th</sup> JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 61<sup>st</sup> JECFA (2003) and published in FNP 52 Add 11 (2003) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI for bixin of 0 – 12 mg/kg bw and a group ADI for norbixin and its disodium and dipotassium salts of 0 – 0.6 mg/kg bw expressed as norbixin were established at the 67<sup>th</sup> JECFA (2006). The colouring matters bixin and norbixin derived from annatto extracts (solvent-extracted bixin; solvent-extracted norbixin; aqueous-processed bixin; alkali-processed norbixin, acid-precipitated; and alkali-processed norbixin, not acid-precipitated) are included in the ADIs for bixin and norbixin. All previous ADIs for annatto extracts were withdrawn.

### SYNONYMS

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

### DEFINITION

Aqueous-processed bixin is prepared by removal of the outer coating of the seeds of the annatto tree (*Bixa orellana* L) by abrading the seeds in the presence of cold, mildly-alkaline water. The resultant preparation is acidified to precipitate bixin which is then filtered, dried and milled.

Aqueous-processed 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

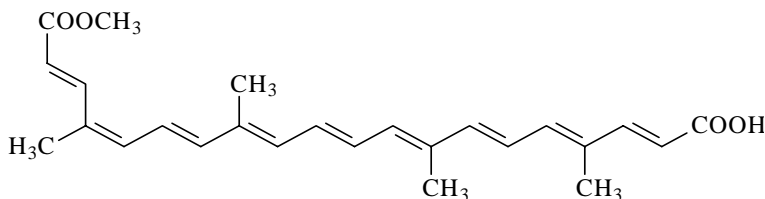
### C.A.S. number

*cis*-Bixin: 6983-79-5

### Chemical formula

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

### Structural formula



*cis*-Bixin

### Formula weight

394.5

### Assay

Not less than 25% 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 sulfuric acid and the spots immediately decolourise.

## PURITY

### Norbixin (Vol. 4)

Not more than 7 % of total colouring matters

### Arsenic (Vol. 4)

Not more than 3 mg/kg  
Determine using an ICP-AES/AAS-Hydride technique. Alternatively, determine arsenic using Method II of the Arsenic Limit Test. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.

### Lead (Vol. 4)

Not more than 2 mg/kg  
Determine using an AAS ICP-AES 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.

### Mercury (Vol. 4)

Not more than 1 mg/kg  
Determine using cold vapour atomic absorption technique. Select sample size appropriate to the specified level.

## 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.