

# ANNATTO EXTRACT (ALKALI-PROCESSED NORBIXIN) (TENTATIVE)

Prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). The previous specifications for annatto extracts (oil and alkali-extracted) prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) have been replaced by these and separate specifications for "Annatto extract (aqueous-processed bixin)", "Annatto extract (alkali-processed norbixin, not acid-precipitated)" and "Annatto extract (oil-processed bixin)". A temporary ADI of 0 – 0.4 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

## SYNONYMS

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

## DEFINITION

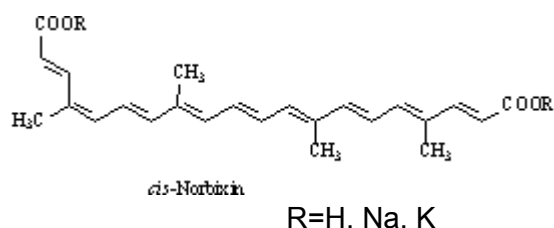
Seeds from the annatto tree (*Bixa orellana* L.) are abraded in cold aqueous alkali (potassium or sodium hydroxide). Additional alkali is added to the resulting suspension which is then heated to dissolve the pigment and cooled. Fats and waxes are removed. The aqueous solution is filtered and acidified to precipitate the norbixin. The precipitate is filtered, washed, dried and milled to give a granular powder. Alkali-processed annatto will contain the potassium or sodium salt of norbixin as the major pigment. Thermal degradation products may also be present as a result of processing. The major colouring principal is *cis*-norbixin. A minor colouring principal is *trans*-norbixin.

Chemical name 9'-*cis*-6,6'-Diapocarotene-6,6'-dioic acid; mono- or di-sodium salt, mono- or di-potassium salt

C.A.S. number *cis*-Norbixin 626-76-6

Chemical formula  $C_{24}H_{28}O_4$ ,  $C_{24}H_{26}Na_2O_4$ ,  $C_{24}H_{26}K_2O_4$

Structural formula



Formula weight 380.48 (acid), 424.45 (sodium salt); 456.68 (potassium salt)

Assay Not less than 35% pigment (expressed as norbixin)

## DESCRIPTION

Dark red-brown to red-purple powder

## FUNCTIONAL USES

Colour

## CHARACTERISTICS

### IDENTIFICATION

Solubility (Vol. 4)

Soluble in alkaline water, slightly soluble in ethanol

UV/VIS absorption  
(Vol. 4)

The sample in 0.5% potassium hydroxide solution shows absorbance maxima at about 453 nm and 482 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

### METHOD OF ASSAY

Proceed as directed in *Colouring matters, Total Content by Spectrophotometry* (Vol. 4), procedure 2 using the following conditions:

$$W = 0.100 \text{ g}$$

$$V_1 = V_2 = V_3 = 100 \text{ ml}$$

$$v_1 = v_2 = 5 \text{ ml}$$

$$A_{1 \text{ cm}}^{1\%} = 2870$$

$$A_{\text{max}} = \text{about } 482 \text{ nm}$$

Use 0.5% potassium hydroxide as solvent