

ANNATTO EXTRACT (SOLVENT-EXTRACTED NORBIXIN) (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for annatto extracts (solvent-extracted) prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) have been replaced by these and separate specifications for "Annatto extract (solvent-extracted bixin)". A temporary ADI of 0 – 0.4 mg/kg bw was established at the 61st 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

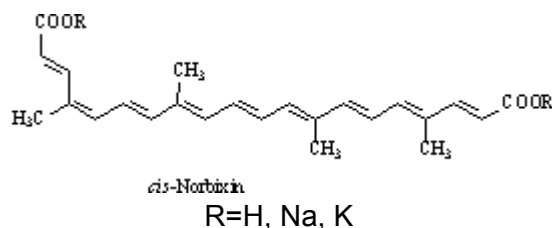
Seeds from the annatto tree (*Bixa orellana* L.) are washed with solvent to dissolve pigment. The extract is filtered to remove insoluble material. Subsequent processing involves removal of fats and waxes, solvent removal, crystallisation and drying. Solvents used can be one or more of: hexane, acetone, ethanol, (alkaline) methanol, isopropanol, ethyl acetate. Aqueous alkali is added to the resultant powder, which is then heated to hydrolyse the pigment and cooled. The aqueous solution is filtered, and acidified to precipitate the norbixin. The precipitate is filtered, washed, dried and milled, to give a granular powder. 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 85% pigment (expressed as norbixin)

DESCRIPTION

Dark red-brown to red-purple powder

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Soluble in alkaline water, slightly soluble in ethanol
<u>UV/VIS absorption</u> (Vol. 4)	The sample in 0.5% potassium hydroxide solution shows absorbance maxima at about 453 nm and 482 nm
<u>Thin Layer Chromatography</u>	Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250 μm , size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10 μl 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

<u>Arsenic</u> (Vol. 4)	Not more than 3 mg/kg
<u>Lead</u> (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".
<u>Mercury</u> (Vol. 4)	Not more than 1 mg/kg
<u>Residual solvents</u>	Acetone: Not more than 30 mg/kg Methanol: Not more than 50 mg/kg Hexane: Not more than 25 mg/kg Ethanol: } Isopropyl alcohol: } Not more than 50 mg/kg, singly or in combination 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:

$$\begin{aligned}W &= 0.100 \text{ g} \\V_1 &= V_2 = V_3 = 100 \text{ ml} \\v_1 &= v_2 = 5 \text{ ml} \\A_{1 \text{ cm}}^{1\%} &= 2870 \\A_{\text{max}} &= \text{about } 482 \text{ nm}\end{aligned}$$

Use 0.5% potassium hydroxide as solvent

