ANNATTO EXTRACT (AQUEOUS-PROCESSED BIXIN)
(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annatto extracts (oil- and alkali-extracted) prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) have been replaced by these and separate specifications for "Annatto extract (alkali-processed norbixin)", "Annatto extract (alkali-processed norbixin, not acid-precipitated)" and "Annatto extract (oil-processed bixin)". A temporary ADI of 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; an appropriate method to determine norbixin at the specified limit.

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

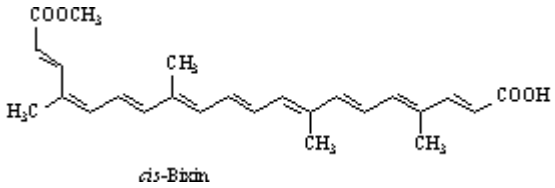
DEFINITION Seeds from the annatto tree (*Bixa orellana* L.) are abraded in cold aqueous alkali (potassium or sodium hydroxide) to remove pigment. The resultant suspension is acidified (sulfuric acid) to precipitate the bixin. The precipitate is filtered, washed dried and milled, to give a granular powder. Thermal degradation products may also be present as a result of processing. The major colouring principal is *cis*-bixin. A minor colouring principal is *trans*-bixin.

Chemical name 9'-*cis*-6,6'-Diapocarotene-6,6'-dioic acid, monomethyl ester

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

Chemical formula $C_{25}H_{30}O_4$

Structural formula



cis-Bixin

Formula weight 394.51

Assay Not less than 25% pigment (expressed as bixin)
Pigment must contain not more than 7% norbixin

DESCRIPTION Dark red-brown to red-purple powder

FUNCTIONAL USES Colour

CHARACTERISTICS

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 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

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 g
$V_1 = V_2 = V_3 = 100$ ml
$v_1 = v_2 = 5$ ml
$A^{1\%}_{1\ cm} = 3090$
A_{max} = about 487 nm

Dissolve sample in 10 ml tetrahydrofuran
Use acetone as solvent