ANNATTO EXTRACTS (SOLVENT-EXTRACTED NORBIXIN)

Prepared at the 80th JECFA and published in FAO JECFA Monographs 17 (2015) superseding specifications prepared at the 67th JECFA (2006) published in FAO JECFA Monographs 3 (2006). A group ADI for norbixin and its disodium and dipotassium salts of 0 – 0.6 mg/kg bw expressed as norbixin was established at the 67th JECFA (2006).

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

DEFINITION Solvent-extracted norbixin is obtained from the outer coating of the seeds of the annatto tree (Bixa orellana L.) by washing with one or more of the following food grade solvents: acetone, methanol, hexane, ethanol, isopropyl alcohol, ethyl acetate, alkaline alcohol or supercritical carbon dioxide followed by solvent removal, crystallization and drying. Aqueous alkali is added to the resultant powder, which is then heated to hydrolyse the colouring matter 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.

Solvent-extracted norbixin contains several coloured components; the major colouring principle is *cis*-norbixin, a minor colouring principle is *trans*-norbixin; thermal degradation products of norbixin may also be present as a result of processing.

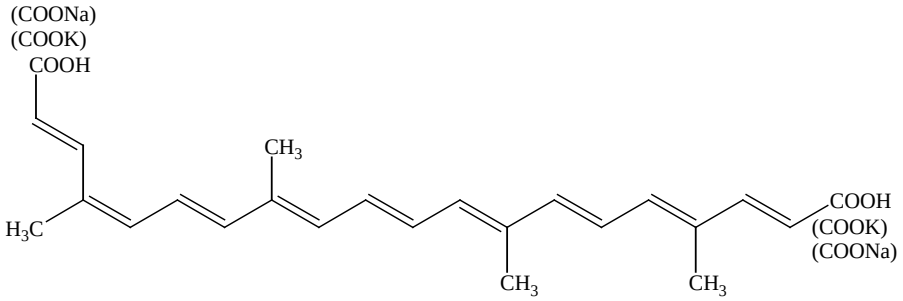
Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name *cis*-Norbixin: 6,6'-Diapo-Ψ,Ψ-carotenedioic acid
cis-Norbixin dipotassium salt: Dipotassium 6,6'-diapo-Ψ,Ψ-carotenedioate
cis-Norbixin disodium salt: Disodium 6,6'-diapo-Ψ,Ψ-carotenedioate

C.A.S. number *cis*-Norbixin: 542-40-5
cis-Norbixin dipotassium salt: 33261-80-2
cis-Norbixin disodium salt: 33261-81-3

Chemical formula *cis*-Norbixin: $C_{24}H_{28}O_4$, *cis*-Norbixin dipotassium salt: $C_{24}H_{26}K_2O_4$, *cis*-Norbixin disodium salt: $C_{24}H_{26}Na_2O_4$

Structural formula



cis-Norbixin

Formula weight 380.5 (acid), 456.7 (dipotassium salt), 424.5 (disodium salt)

Assay Not less than 85 % colouring matter (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 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

Residual Solvents

Acetone:	Not more than 30 mg/kg
Methanol:	Not more than 50 mg/kg
Hexane:	Not more than 25 mg/kg
Ethanol: Isopropyl alcohol: Ethyl acetate:	Not more than 50 mg/kg, singly or in combination

See Description under TEST

Arsenic (Vol. 4) Not more than 3 mg/kg
Determine using an AAS (Hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Mercury (Vol. 4) Not more than 1 mg/kg
Determine using AAS (Cold vapour generation technique). The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 1, using 0.5 % potassium

hydroxide as solvent. Measure the absorbance at the A_{max} of about 482 nm. The specific absorbance ($A_{1cm}^{1\%}$) is 2870.

TESTS

Residual solvents

Proceed as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

Stock standard solution
Add 10 ml dimethylformamide to a 20 ml volumetric flasks. Accurately weigh, to within 0.01 mg, each flask. Pipet 250 µl each of chromatography grade methanol, ethanol, isopropanol, and ethyl acetate, and 150 µl each of acetone and hexane into each of the flask. Reweigh accurately and then fill the flask with dimethylformamide. Mix well.

Standard mixture solution A: Pipet each 3.0 ml of stock standard solution into a 20 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution B: Pipet 4.0 ml solution A into a 10 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution C: Pipet 2.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution D: Pipet 1.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Samples
Weigh accurately 0.2 g sample into a 20 ml injection vial. Add 2.5 ml dimethylformamide and seal.

Standard solutions
Introduce 0.1 ml of the each standard mixture solution (A, B, C and D) into each 20 ml injection vial. Add 2.4 ml dimethylformamide and seal.

Standard curves
Place the four standard solutions in the sample tray on head-space gas chromatography. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical condition as described above. Measure the peak area for each solvent. Construct the standard curves by plotting the ratios of the peak areas of each solvent against the concentrations of each solvent (mg/ml) in the standards solutions.

Procedure
Place the sample solution in the sample tray on head-space gas chromatograph. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4.
Measure the peak area for each solvent and obtain the concentration of each solvent (C, mg/ml) from the standard curves.

Calculation
Calculate the concentration of each residual solvent in samples from;

$$\text{Residual solvent (mg/kg)} = C \times 2.5/W \times 1000$$

Where:
W is weight of sample (g).