MIXED CAROTENOIDS

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). No ADI was allocated at the 31st JECFA (1987)

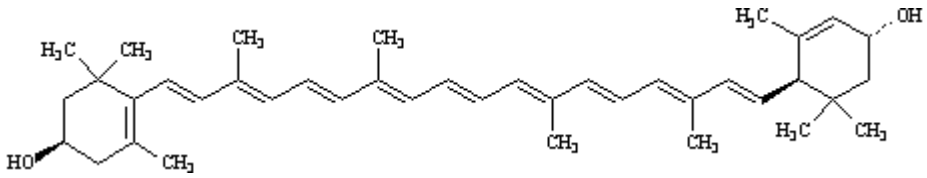
DEFINITION Mixed carotenoids are obtained by solvent extraction of alfalfa, removal of chlorophylls through saponification and subsequent purification of the carotenoids by solvent extraction. The main colouring principle consists of carotenoids of which lutein accounts for the major part. Variable amounts of neoxanthin, violaxanthin and ß-carotene are present.
Mixed carotenoids may contain fats, oils and waxes naturally occurring in the plant material. Vegetable oils may be added for standardizing purposes. Only the following solvents may be used for the extraction: methanol, ethanol, propan-2-ol, hexane, acetone, dichloromethane and methyl ethyl ketone. (Note: Articles of commerce may contain approved food additives in order to formulate water soluble products.)

Chemical name: Lutein: 3,3'-dihydroxy-d-carotene; ß,epsilon-carotene-3,3'-diol

C.A.S. number: Lutein: 127-40-2

Chemical formula: Lutein: $C_{40}H_{56}O_2$

Structural formula:



Formula weight: Lutein: 568.88

Assay: Total colouring matter (as lutein) not less than declared

DESCRIPTION Dark, yellowish brown liquids with a weak hay-like odour

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water; soluble in hexane.

Spectrophotometry (Vol. 4): A chloroform solution of the sample shows maximum absorption at about 445 nm

Carr-Price test: A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price TS.

PURITY

Synthetic colours — Samples should be free of synthetic pigments, canthaxanthin, and apocarotenoic acid ethyl ester.
See description under TESTS

Residual solvent (Vol. 4) — Acetone, methanol, ethanol, propan-2-ol, and hexane, not more than 50 mg/kg, singly or in combination.
Dichloromethane and methyl ethyl ketone, not more than 10 mg/kg, individually.

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Synthetic colours — Unknown samples are compared to authentic alfalfa carotenoids. Ground dried alfalfa is extracted with dichloromethane. The extract is saponified and the carotenoids separated by reverse phase liquid chromatography. This test is qualitative and is intended to differentiate alfalfa mixed carotenoids from artificial colours.

Apparatus
High pressure liquid chromatograph (HPLC) equipped with a variable wavelength detector, a Zorbax ODS 150 mm x 4.6 mm id reverse phase column or equivalent, and a suitable 10-µl injection value.

Reagents
Acetonitrile, dichloromethane, ethyl acetate, acetone (all HPLC grade), n-decanol, butylated hydroxytoluene (BHT), potassium hydroxide, methanol, sodium sulfate (all analytical reagent or better grade). Add 1 g/l of BHT to all solvents. Add 0.1% (v/v) n-decanol to all mobile phases.

Chromatography and detection
Mobile phase: ethyl acetate: acetonitrile (12:88) 1.6 ml/min; injection volume 10 µl; detector wavelength 450 nm, full scale range 0.16 AUFS.

Procedure
Grind dried alfalfa to pass 1-mm mesh screen and thoroughly mix. Accurately weigh 1-2 g sample in a glass stoppered boiling tube. Add 30 ml dichlormethane: acetone (2:1) and shake. Let stand overnight. Add 20 ml dichloromethane, 2 ml 40% (w/v) methanolic potassium hydroxide, shake, and let stand 60 min. Add 30 ml of 10% (w/v) aqueous sodium sulfate, shake, and let stand 60 min. Remove an aliquot of the lower layer and centrifuge 10 min at 2000 rpm. Remove a 3-ml aliquot of the lower yellow layer, mix with 3 ml acetonitrile, and use this solution for analysis. Unknown mixed carotenoid samples are prepared by dissolving a 0.1-0.2 g sample in 50 ml of dichloromethane:acetonitrile (1:1). Inject 10 µl of alfalfa mixed

carotenoid standard followed by 10 µl of unknown mixed carotenoid sample and compare chromatograms.

The approximate retention times for neoxanthin, violaxanthin, lutein, zeaxanthin, alpha-carotene, and ß-carotene are 3.5, 3.9, 6.0, 7.0, 18, and 19 min, respectively. Synthetic pigments, canthaxanthin, and apocarotenoic acid ethyl ester elute just before lutein.

METHOD OF ASSAY

Proceed as directed in Volume 4, *General Methods*, Methods for Food Colours, Colouring Matters, Total Content by Spectrophotometry, Procedure 2, using the following conditions:

W = amount (g) to obtain adequate absorbance
$V_1 = V_2 = V_3 = 100$ ml
$v_1 = v_2 = 10$ ml
$A^{1\%}_{1cm} = 2500$
lambda $_{max}$ = app. 445 nm