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FAO JECFA Monographs

22

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

86th Meeting 2018

Food and Agriculture Organization of the United Nations

World Health Organization

FAO JECFA Monographs 22

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Geneva, 12 – 21 June 2018

Food and Agriculture Organization of the United Nations
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Geneva, 2018

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Geneva, 12 – 21 June 2018

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 86th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva, 12 – 21 June 2018. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consists of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings specifications. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 79th meeting, are available in the online searchable database at the JECFA website at FAO: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/en/. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

An account of the purpose and function of specifications of identity and purity, the role of JECFA specifications in the Codex system, the link between specifications and methods of analysis, and the format of specifications, are set out in the Introduction to the Combined Compendium, which is available in shortened format online on the query page, which could be consulted for further information on the role of specifications in the risk assessment of additives.

Chemical and Technical Assessments (CTAs) for some of the food additives have been prepared as background documentation for the meeting. These documents are available online at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/technical-assessments/en/.

Contact and Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/. Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

jecfa@fao.org

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for 11 food additives and these are presented in this publication.

Anionic methacrylate copolymer (AMC) (N, T)
Basic methacrylate copolymer (BMC) (N)
Cassia gum (R)
Citric and fatty acid esters of glycerol (R, T)
Erythrosine (R)
Glycerol ester of wood rosin (R)
Indigotine (R)
Lutein (R)
Modified starches[1] (R, T)
Neutral methacrylate copolymer (NMC) (N, T)
Spirulina extract (N)

In the specifications monographs that have been assigned a tentative status (T), there is information on the outstanding data and a timeline by which this information should be submitted to the FAO JECFA Secretariat.

[1] Applying to all 16 modified starches: INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451

ANIONIC METHACRYLATE COPOLYMER

New specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). No ADI was established at the 86th JECFA (2018).

SYNONYMS E 1207, INS No. 1207, acrylates copolymers, Methyl acrylate, methyl methacrylate, methacrylic acid polymer; methacrylic acid, polymer with methyl acrylate and methyl methacrylate

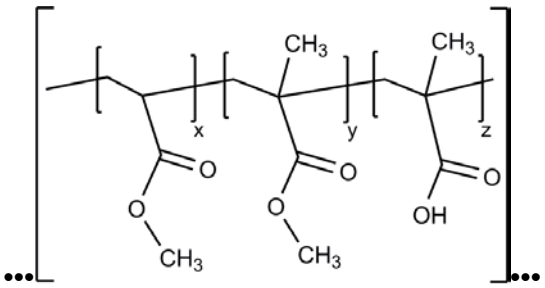
DEFINITION Anionic methacrylate copolymer is a copolymer comprised of monomers, methyl acrylate, methyl methacrylate, and methacrylic acid in the molar ratio of 7:3:1. The copolymer is manufactured by emulsion polymerization of the monomers with water soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low-molecular weight substances and coagulum. The copolymer is standardized as a 30% aqueous dispersion. The copolymer dispersion may contain residual monomers (methyl acrylate, methyl methacrylate, and methacrylic acid). Anionic methacrylate copolymer is used as a coating and glazing agent
for food supplements and products for special medical purposes.

Chemical name Poly (methyl acrylate-co-methylmethacrylate-co-methacrylic acid) 7:3:1

C.A.S. number 26936-24-3

Chemical formula Poly[(CH_2:$CHCO_2CH_3$)-co-(CH_2:$C(CH_3)CO_2CH_3$)-co-(CH_2:$C(CH_3)COOH$)]

Structural formula



The above formula is provided for illustrative purposes; in this copolymer no definitive structural unit can be defined.

Formula weight 280,000 (weight-average), 77,000 (number-average)

Assay 9.2 – 12.3 % methacrylic acid units on the dried basis

	See description under Tests
DESCRIPTION	Commercial form (30% aqueous dispersion) is a low viscosity, milky-white liquid.
FUNCTIONAL USES	Coating agent, glazing agent.
CHARACTERISTICS	
IDENTIFICATION	
Viscosity (Vol. 4)	Not more than 20 mPa•s Determine viscosity using Brookfield viscometer at 20° and 30 rpm using UL adapter.
pH (Vol 4)	2.0 – 3.5
Infrared absorption (Vol. 4)	The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix. Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.
PURITY	
Loss on drying (Vol. 4)	68.5 – 71.5% (110°, 5 h)
Sulfated ash (Vol. 4)	Not more than 0.2% Test 5 g of the sample (Method I)
Methanol (Vol. 4)	Not more than 1,000 mg/kg
Residual monomers	Methyl acrylate: Not more than 1 mg/kg Methyl methacrylate: Not more than 3 mg/kg Methacrylic acid: Not more than 1 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 1.0 mg/kg in the dispersion

Determine using a method 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”).

Microbiological criteria (Vol. 4)

Total plate count: Less than 1,000 cfu/g

Yeast and moulds: Less than 100 cfu/g

Coliforms: Negative in 10 g

TESTS

IDENTIFICATION TESTS

Residual monomers

Determined by liquid chromatography (Vol. 4)

Standards and Reagents:

- Acetonitrile: HPLC grade with UV absorption: A_{max} of 1% at 190 nm
- Acetone, methanol, isobutanol and deionized water: HPLC grade
- Phosphoric acid solution (pH 2): Adjust phosphoric acid (85 %) with an appropriate volume of deionized water to pH 2.
- Standards: methyl acrylate, methyl methacrylate and methacrylic acid (>99%)

Preparation of mixed standard solutions:

Stock mixed standard solution:

Pipette 5 ml of isobutanol into a 50 ml volumetric flask. Accurately weigh approximately 10 mg of methyl acrylate, 12 mg of methyl methacrylate and 11 mg of methacrylic acid, add to isobutanol and dilute to volume with acetone.

Intermediate mixed standard solution-1:

Dilute 5.0 ml of stock mixed standard solution to 50 ml with acetone in a volumetric flask.

Intermediate mixed standard solution-2: Dilute 20.0 ml of intermediate mixed standard solution-1 to 50 ml with acetone in a volumetric flask.

Working mixed standard solution:

Dilute 5 ml of Intermediate mixed standard solution-2 to 25 ml with methanol:phosphoric acid-pH 2 (70:30) in a volumetric flask.

Preparation of sample solution:

Accurately weigh approximately 11 g of sample, dissolve in acetone and dilute to 50 ml in a volumetric flask. Add 5.0 ml of

the solution dropwise (precipitation of the polymer should be slow to avoid entrapment of monomer in the precipitate) to 20 ml methanol and phosphoric acid- pH 2 (70:30 v/v). Centrifuge until the supernatant is clear and use the supernatant as the sample solution.

Procedure:

- Use an HPLC with diode array or UV detector at 200 nm
- Column: Octadecylsilane chemically bonded to porous silica (125 cm x 4.6 mm i.d.x 7 µm)
- Injection volume: 20 µl
- Mobile phase: Acetonitrile:Phosphoric acid-pH 2 (10:90 v/v)
- Flow rate: 2 ml/min

Inject separately 20 µl each of working mixed standard solution and sample solution. Calculate the amount of each monomer in the sample from the peak areas obtained in the chromatograms of working mixed standard solution (rR) and sample solution (rS); amount of standard (R, mg), weight of sample (W, g) and dilution factor (40).

$$Conc.monomer\ [\mu g/g] = \frac{rS\ \times R\ \times 40}{rR\ \times\ W}$$

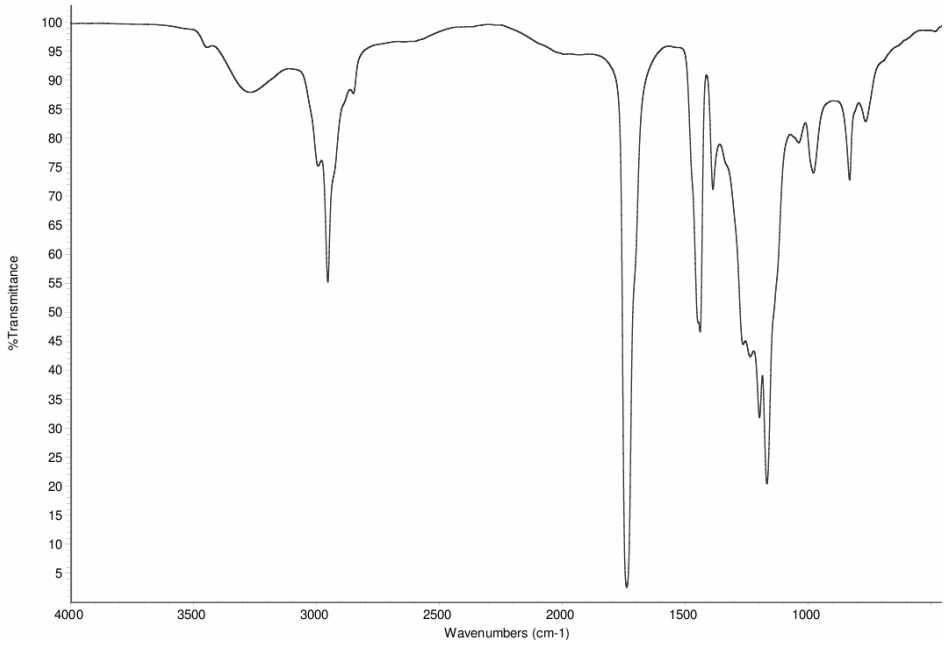
Total monomers in the sample (µg/g) = Sum of monomers in the sample and correct the results for recovery.

METHOD OF ASSAY

Accurately weigh about 5 g sample and dissolve completely in 90 ml isopropyl alcohol and 10 ml water. Titrate with 0.5 N sodium hydroxide standard solution to a potentiometric endpoint. Perform a blank titration under the same conditions. One ml 0.5 N NaOH corresponds to 43.045 mg methacrylic acid units.

$$Methacrylic\ acid\ units\ (\%w/w, on\ the\ dried\ basis) = \frac{ml\ of\ 0.5\ N\ NaOH \times 430.45}{sample\ weight\ (g) \times \%\ dry\ substance\ in\ sample}$$

Appendix: Infrared spectrum of anionic methacrylate copolymer



BASIC METHACRYLATE COPOLYMER

New specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). An ADI of "not specified" was established at 86th JECFA (2018).

SYNONYMS E 1205; INS No. 1205; basic butylated methacrylate copolymer; amino methacrylate copolymer; aminoalkyl methacrylate copolymer E; butyl methacrylate, dimethylaminoethyl methacrylate, methyl methacrylate polymer; butyl methacrylate, methyl methacrylate, dimethylaminoethyl methacrylate copolymer

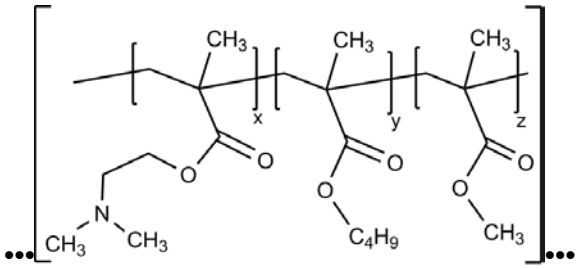
DEFINITION Basic Methacrylate Copolymer is a cationic copolymer comprised of the monomers dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate in the molar ratio of 1:2:1. The copolymer is manufactured by a controlled polymerization process using a free radical donor initiation system. After completion of polymerization, the viscous copolymer solution is fed into an extruder to remove solvents and volatile substances, by actively degassing through vacuum and heating. The solid granules of basic methacrylate copolymer formed in the extruder can be milled to a powder. The copolymer may contain residual monomers (dimethylaminoethyl methacrylate, butyl methacrylate, methyl methacrylate,). Basic methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes.

Chemical name Poly(butyl methacrylate-*co*-(2-dimethylaminoethyl)methacrylate-*co*-methyl methacrylate)

C.A.S. number 24938-16-7

Chemical formula Poly[(CH_2:$C(CH_3)CO_2(CH_2)_2N(CH_3)_2$)-*co*-($CH_2$:$C(CH_3)CO_2CH_3$)-*co*-($CH_2$:$C(CH_3)CO_2(CH_2)_3CH_3$)]

Structural formula



The above formula is provided for illustrative purposes; specific repeat units cannot be defined.

Formula weight 47,000 (weight-average), 22,000 (number-average)

Assay	20.8 – 25.5 % dimethylaminoethyl (DMAE) groups on the dried basis See description under TESTS
DESCRIPTION	White powder
FUNCTIONAL USES	Coating agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Viscosity	3 - 6 mPa·s Determine viscosity using Brookfield viscometer at 20° and 30 rpm using UL adapter.
Refractive index (Vol. 4)	n_D^{20}: 1.380 - 1.385
Solubility (Vol. 4)	Freely soluble in methanol, ethanol, and 1 N aqueous hydrochloric acid
Infrared absorption	The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix. Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol. 4)	Not more than 2.0% (110°, 3 h)
Sulfated ash (Vol. 4)	Not more than 0.1% Test 5 g of the sample (Method I)
Particle size	< 50 µm: at least 95 % < 20 µm: at least 50 % < 3 µm: not more than 10 % See description under TESTS
Residual solvents (Vol. 4)	Methanol: Not more than 50 mg/kg; Butanol: not more than 50 mg/kg and

Propan-2-ol: not more than 100 mg/kg.

(See General Methods, Organic Components, Residual Solvents, Method 1)

Residual monomers — Dimethylaminoethyl methacrylate: Not more than 500 mg/kg

Butyl methacrylate: Not more than 100 mg/kg

Methyl methacrylate: Not more than 50 mg/kg

See description under TESTS

Lead (Vol. 4) — Not more than 1.0 mg/kg

Determine using a method 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").

Microbiological criteria (Vol. 4) — Total plate count: Not more than 1,000 cfu/g

Yeast and moulds: Not more than 100 cfu/g

Coliforms: Negative in 10 g

TESTS

PURITY TESTS

Particle size — Determine using the light diffraction measurement method according to Ph. Eur. 2.9.31

(European Pharmacopeia; Particle Size Analysis by Laser Light Diffraction. 8 01/2010:0333)

Residual monomers — **Method for the determination of methyl methacrylate and butyl methacrylate:**

Standards and Reagents:

Phosphate buffer (0.0625 M, pH 2.0):

Prepare an aqueous solution containing 8.9 g of anhydrous dibasic sodium phosphate and 8.5 g of monobasic potassium phosphate in 1 L deionized water. Adjust with phosphoric acid to pH 2.0.

Mobile phase:

Prepare a mixture of methanol and pH 2.0 phosphate buffer (55:45).

Diluent:

Acetonitrile:Buffer (40:60)

Preparation of standard solution

Stock mixed standard solution:

Accurately weigh 20 mg of butyl methacrylate and 10 mg of methyl methacrylate, dissolve in 3 ml of n-butanol and dilute to volume to 10 ml with diluent in a volumetric flask.

Intermediate mixed standard solution:

Pipette 1.0 ml of stock mixed standard solution into a 10 ml volumetric flask and dilute to 10 ml with diluent.

Working mixed standard solution:

Pipette 1.0 ml of intermediate standard solution into a 25 ml volumetric flask and dilute to volume with diluent. This solution contains about 8 µg/ml of butylmethacrylate and 4 µg/ml of methyl methacrylate.

Preparation of Sample solution

Accurately weigh about 1.0 g of sample, dissolve in diluent and make up to 50 ml with diluent and mix.

Procedure

Chromatographic system:

The liquid chromatograph is equipped with a UV/diode array detector capable of working at 205 nm and a column (4.6 mm × 12 cm, packing material: octadecylsilane chemically bonded to porous silica or ceramic microparticles, 1.5-10 µm)). Flow rate: 2 ml/min.

Chromatograph the working standard solution, and record the peak responses. The resolution, R, between butyl methacrylate and methyl methacrylate is not less than 10; and the relative standard deviation for replicate injections is not more than 3.0%.

Separately inject 50 µl each of the working standard solution and sample solution and record the peak areas of the monomers.

Calculate the quantity of each monomer in the sample using the formula:

$$\text{Monomer concentration } (\mu g/g) = (rU/rS) \times (CS/CU) \times F$$

rU = Peak area for the monomer in the sample chromatogram

rS = Peak area for the monomer in the working standard chromatogram

CS = Concentration of monomer in the working standard solution (µg/ml)

CU = Concentration of polymer in the sample solution (mg/ml)

F = Conversion factor (10^3 mg/g)

Method for the determination of 2-Dimethylaminoethyl methacrylate

Standards and Reagents:

Monobasic potassium phosphate buffer solution (0.025M):

Prepare an aqueous solution containing 3.4 g of monobasic potassium phosphate per litre.

Mobile phase:

Tetrahydrofuran:monobasic potassium phosphate buffer solution (75:25).

Preparation of standard solution

Stock standard solution (200 µg/ml):

Accurately weigh about 20 mg of (2-dimethylaminoethyl) methacrylate, dissolve in tetrahydrofuran, make up to volume in a 10 ml volumetric flask with tetrahydrofuran and mix.

Working standard solution (8 µg/ml):

Dilute 2.0 ml of the stock standard solution to 50 ml in a volumetric flask with tetrahydrofuran and mix.

Preparation of Sample solution

Accurately weigh about 1.0 g of sample, dissolve in tetrahydrofuran, dilute to 50 ml with tetrahydrofuran in a volumetric flask and mix.

Procedure

Chromatographic system:

The liquid chromatograph is equipped with a UV/diode array detector capable of working at 215 nm and a column (4.6 mm × 12 cm, packing material: an essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5-10 µm) . Flow rate: 2 ml/min.

Chromatograph the working standard solution, and record the peak area. The relative standard deviation for replicate injections is not more than 2.0%.

Separately inject 50 µl of the working standard solution and the sample solution and record the peak areas.

Calculate the quantity of each monomer in the sample using the formula"

$$\text{2-Dimethylaminoethyl methacrylate, (µg/g)} = (rU/rS) \times (CS/CU) \times F$$

rU = Peak area for the monomer in the sample chromatogram

rS = Peak area for the monomer in the standard chromatogram

CS = Concentration of monomer in the working standard solution (µg/ml)
CU = Concentration of polymer in the sample solution (mg/ml)
F = Conversion factor (10^3 mg/g)

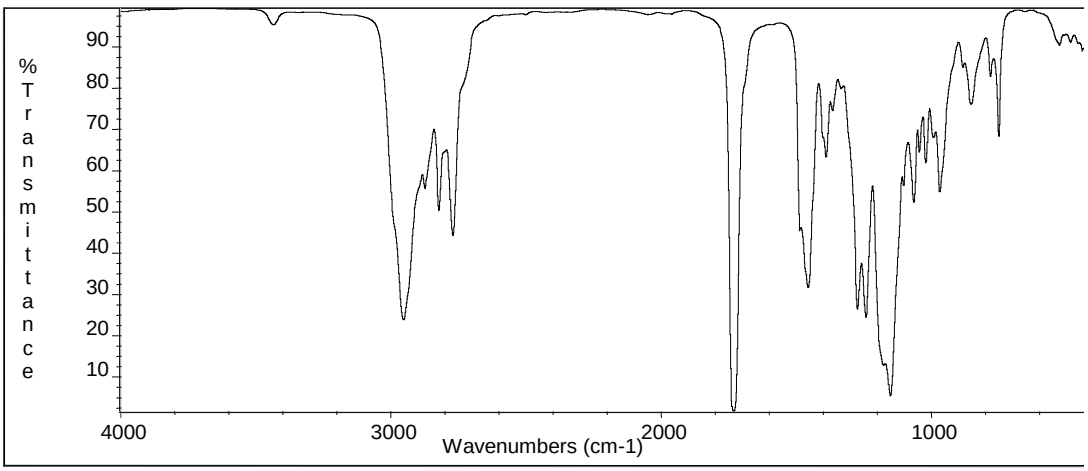
METHOD OF ASSAY Determine the percentage of Dimethylaminoethyl (DMAE) groups using a potentiometric titration.

Dissolve 200 mg of dried sample in 4 ml water and 96 ml of glacial acetic acid. Titrate with 0.1 N standard perchloric acid solution to a potentiometric end point. Perform a blank determination.

$$DMAE\ groups\ (\%w/w, on\ the\ dried\ basis) = \frac{(VS - VB)\ \times N\ \times\ F}{W}\ \times\ 100$$

VS = titrant volume consumed by the sample (ml)
VB = titrant volume consumed by the blank (ml)
N = actual normality of the titrant (mEq/ml)
F = equivalency factor, 72.1 mg/mEq
W = dried sample weight (mg)

Appendix**:** Infrared spectrum of basic methacrylate copolymer



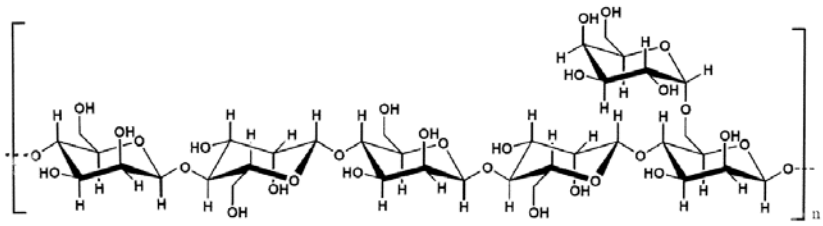
CASSIA GUM

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018), superseding tentative specifications prepared at the 82nd JECFA (2016) and published in FAO JECFA Monographs 19 (2016). An ADI "not specified" was established at the 71st JECFA (2009)

SYNONYMS INS 427

DEFINITION Cassia gum is obtained from the ground purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia* (Fam. *Leguminosae*) containing less than 0.05% of *Cassia occidentalis*. It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 5:1. The seeds are dehusked and degermed by thermal and mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is purified by extraction with isopropanol.

Structural formula



The structure above is provided for illustrative purposes. A specific repeat unit for Cassia gum cannot be defined.

Assay Not less than 75% of galactomannans

DESCRIPTION Pale yellow to off-white, odourless free-flowing powder. Forms colloidal solutions in cold water.

FUNCTIONAL USES Thickener, emulsifier, foam stabilizer, moisture retention agent, and texturizing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility Insoluble in ethanol

Gel formation with borate Add sodium borate TS to an aqueous dispersion of the sample to raise the pH above 9; a gel is formed.

Gel formation with xanthan gum	Passes test See description under TESTS
Gum constituents (Vol. 4)	Proceed as directed under 'Gum Constituents Identification' using 100 mg of sample (instead of 200 mg) and 1-10 µl of the hydrolysate (instead of 1-5 µl). Use galactose and mannose as reference standards. These constituents should be present.
Viscosity	Less than 500 $\mathrm{mPa} \times \mathrm{s}$ See description under TESTS
pH (Vol. 4)	5.5-8.0 (1% solution)
PURITY	
Loss on drying (Vol. 4)	Not more than 12% (105º , 5 h)
Total ash (Vol. 4)	Not more than 1.2%
Acid-insoluble matter (Vol. 4)	Not more than 2.0%
Protein (Vol. 4)	Not more than 7.0% Multiply percent nitrogen by 6.25.
Crude fat	Not more than 1% See description under TESTS
Starch	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
Anthraquinones	Not more than 0.5 mg/kg See description under TESTS
Residual solvents (Vol. 4)	Isopropanol: Not more than 1.0% See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method 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 (under "General Methods, Metallic Impurities").

Microbiological criteria (Vol. 4)

Total plate count: Not more than 5,000 cfu/g

Yeast and mould: Not more than 100 cfu/g

E. coli: Negative in 1 g

Salmonella: Negative in 25 g

TESTS

IDENTIFICATION TESTS

Gel formation with xanthan gum

Weigh 1.5 g of sample, 1.5 g of xanthan gum and blend them. Add this blend (with rapid stirring) to 300 ml water at 80º in a 400 ml beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60º during the stirring period). Discontinue stirring and allow the mixture to cool to room temperature for at least 2 h.

A firm, viscoelastic gel forms after the temperature drops below 40º, but no such gel forms in a 1% control solution of cassia gum or xanthan gum alone prepared in a similar manner.

Viscosity

Add 495 ml of deionized water into a 1L beaker, insert a magnetic stir bar and place the beaker on a magnetic stirrer equipped with a heater. Adjust the stirrer speed to about 750 rpm. Weigh 5 g of sample and quickly add to the beaker. Switch on the heater and heat the beaker to reach 90º and keep it at 90º for 15 min. Cool the solution to room temperature (25º ±1.5º) in a water bath. Measure the viscosity at 25º, after 2 h, using a RVT Brookfield Spindle 1 and 20 rpm speed. Repeat the procedure with a sample of 5 g of carob (locust) bean gum. The viscosity of the cassia gum (150 – 500 $\mathrm{mPa} \times \mathrm{s}$) must be less than 50% that of carob bean gum (2000 - 3500 $\mathrm{mPa} \times \mathrm{s}$)

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Vol. 4; Method I) under the following conditions.

Internal standard solution

Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl of 3-methyl-2-pentanone through the septum and reweigh the vial to within 0.01 mg.

Standard solution
Add 50.0 ml water to a 50 ml vial and seal weigh accurately. Inject 15 µl isopropanol and reweigh the vial.

Blank solution:
Add 5.0 ml of water and pipette 1.0 ml of the internal standard solution into a headspace vial. Seal the vial and mix the contents using a vortex mixer.

Calibration solution:
Add 4.0 ml of water into the headspace vial. Pipette 1.0 ml each of the internal standard solution and the standard solution. Seal the vial and mix the contents using a vortex mixer.

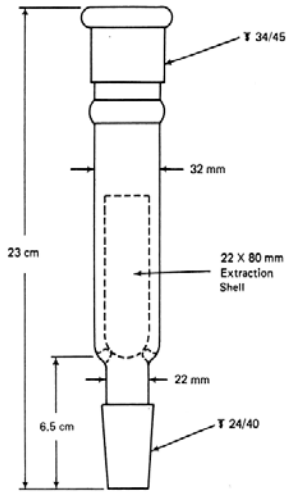
Preparation of sample:
Pipette 5 ml of water and 1 ml internal standard solution into a headspace vial. Accurately weigh 0.500 ± 0.001 g of sample in a small weighing boat and add the sample carefully to prevent clumping of sample at the bottom of the vial. Seal the vial and mix the contents using a vortex mixer. Do not shake the sample vial.

Follow the procedure described in Vol. 4 for the determination of residual solvents.

Crude fat

Apparatus
The apparatus consisting of a Butt-type extractor, as shown below, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-ml Erlenmeyer flask.

Procedure
Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a cellulose thimble or a 15-cm filter paper (roll the paper tightly around the sample), and place it in a suitable extraction shell). Plug the top of the thimble or the extraction shell with cotton previously extracted with hexane, and place it in the extractor. Attach the extractor to a dry 125-ml Erlenmeyer flask containing about 50 ml of hexane and to a water-cooled condenser, apply heat to the flask to produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few ml of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odour of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.



Butt-Type Extractor for fat determination.

NOTE: The method for crude fat is referenced from Appendix X: Crude fat in the Food Chemicals Codex, 11th Edition, 2018. Reproduced from USP-NF with permission from The U.S. Pharmacopeial Convention (USP)

Anthraquinones

Principle

Anthraquinones are extracted with chloroform and determined by High Performance Liquid Chromatography (Vol.4) using the conditions below.

NOTE: Anthraquinones are photosensitive. Samples and standards shall be protected from light and all manipulations shall be carried out under the subdued light.

Standards and Reagents:

Emodin, Aloe-emodin, Physcion (1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone), Rhein and Chrysophanic acid (>99%).
Internal standard: Danthrone (1,8-dihydroxy-anthraquinone, >99%)

Methanol, acetonitrile, deionized water, chloroform, trifluoroacetic acid, sulfuric acid and sodium hydrogen carbonate

Individual stock standard and internal standard solutions (100 µg/ml)

Accurately weigh about 10 mg of the standards and internal standard, transfer to 100 ml volumetric flasks with about 5 ml of methanol, sonicate for 15 min and dilute to volume with methanol.
Store these solutions in amber coloured bottles at 4º (the solutions are stable for 2 weeks under these conditions).

Internal spike standard solution (20 µg/ml)

Dilute 2 ml of internal standard stock solution to 10 ml with methanol.

Mixed standard solution (10 µg/ml)

Transfer 1 ml of each of the anthraquinones stock standard solution into a 10 ml volumetric flask and dilute to volume with methanol.

Working standard solutions

To each of five 10 ml volumetric flasks transfer 0, 0.5, 1, 2 and 5 ml respectively of the mixed standard solution, and 1 ml of the internal spike standard solution (20 µg/ml), dilute to volume with methanol and mix.

Sample preparation:

Accurately weigh about 4.0 g of the sample into a 250 ml Erlenmeyer flask. Add 80 µl of internal standard solution (100 µg/ml), and 100 ml 2N H_2SO_4 to the flask. Stopper the flask using a PTFE stopper and heat at 103º for 3.5 hours in an oven. After cooling to room temperature, add 100 ml of chloroform and shake well. Allow phase separation. Evaporate 50 ml of the chloroform layer to dryness in a rotary evaporator at 68º. Dissolve the residue in 2 ml of methanol. Filter the solution through a PTFE membrane syringe filter.

Chromatographic conditions:

Column: Hypersil ODS C18 (250 mm x 4.6 mm ID, 5 µm)

Mobile phase:

(A) 0.1 % trifluoroacetic acid in water

(B) Acetonitrile

Injection volume: 20 µl

Gradient:

Time, min	% (A)	% (B)
0	86	14
10	86	14
15	80	20
25	80	20
55	20	80
60	0	100
66	86	14

Flow rate: 1 ml/min

Detector: Photodiode Array/UV Detector operated at 435 nm.

Procedure

Inject individual standard solutions and internal standard solution (dilute, if required) and record retention times.

Construction of standard curves

Inject 20 µl of each working standard solution. Construct the standard curves by plotting the ratios of the peak areas of each of the specific anthraquinone / internal standard against the concentrations of each working standard solution (µg/ml).

Inject 20 µl of the Sample solution. Calculate the ratios of the peak areas of each anthraquinone / internal standard, and obtain the amount (A) of each anthraquinone from the respective standard curve.

Concentration of anthraquinone in the sample $(\mu g/g) = (A \times 4)/W$

Where

A = the amount of each anthraquinone (µg) obtained from the standard curve

W = Mass of sample (g)

4 = Dilution factor for sample

METHOD OF ASSAY

$$\%\,\text{Galactomannans} = 100 - (L + A + I + P + F)$$

L % Loss on Drying

A %Total Ash

I %Acid-Insoluble Matter

P %Protein

F %Crude Fat

CITRIC AND FATTY ACID ESTERS OF GLYCEROL (TENTATIVE)

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018), superseding specifications prepared at the 82nd JECFA (2016), and published in FAO JECFA Monographs 19 (2016). An ADI 'not limited' was established at the 17th JECFA (1973)

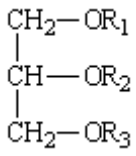
Information required:

- *A validated method for the determination of total citric acid content*
- *Performance characteristics (method validation data) of the citric acid determination method*
- *Data on the total citric acid content, in at least five batches of products currently available in commerce, determined using the above method.*

SYNONYMS Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

DEFINITION Citric and fatty acid esters of glycerol (CITREM) consists of mixed esters of citric acid and edible fatty acids with glycerol. It may contain free fatty acids, glycerol, citric acid and mono- and diglycerides, in minor quantities. The mono- and di- glycerides may include either one or two edible fatty acids from C12:0 to C18:0, mainly the saturated palmitic (C16:0) and stearic (C18:0) acids. It may also contain minor amounts of other fatty acids such as myristic (C14:0), oleic (C18:1), linoleic (C18:2) and arachidic acid (C20:0). CITREM is obtained by esterification of glycerol with citric acid and edible fatty acids, or by reaction of a mixture of mono- and diglycerides of edible fatty acids, with citric acid. CITREM may be partially or wholly neutralized with sodium hydroxide or potassium hydroxide.

Structural formula



Where at least one of R_1, R_2 or R_3 represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION White to ivory coloured, oily to waxy material.

FUNCTIONAL USES Stabilizer, emulsifier, dough conditioner, antioxidant synergist

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water; soluble in oils and fats; insoluble in ethanol
Test for fatty acids (Vol. 4)	Passes test
Test for citric acid	Information required
Test for glycerol (Vol. 4)	Passes test

PURITY

Sulfated ash (Vol. 4)	Non-neutralized products: not more than 0.5% Partially or wholly neutralized products: not more than 10%; test 2 g of the sample (Method I)
Free glycerol (Vol. 4)	Not more than 4%
Total glycerol	8-33% See description under TESTS
Total citric acid	13-50% *(Information required)*
Total fatty acid	37-81% See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg. (Not more than 0.1 mg/kg for use in infant formula and formula for special medical purposes intended for infants) Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Total glycerol

CITREM is hydrolyzed, glycerol in the aqueous phase is oxidized using known excess of sodium periodate in a strongly acid medium and the unreacted periodate is back titrated using standard sodium thiosulfate solution.

Procedure:
Accurately weigh about 2 g of the sample into a saponification flask, add 50 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min.

To a 1-L volumetric flask add 99 ml ± 0.2 ml of chloroform using a burette and add 25 ml of glacial acetic acid using a graduated cylinder. Quantitatively transfer the content of the saponification flask to the volumetric flask, using three 25 ml portions of water. Add about 500 ml of water further, and shake vigorously for about 1 min. Dilute to volume with water, stopper, mix thoroughly and set aside for separation of layers.

Pipet 50 ml of acetic periodic acid TS into a series of 400 ml beakers. Prepare two blanks by adding 50 ml of water to each. Pipet 50 ml of the aqueous layer into one of the 400 ml beakers containing 50 ml of acetic periodic acid TS; shake gently to mix; cover with watch glass, and allow to stand 30 min but not longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 200 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodine-starch complex colour from the aqueous layer. Read the burette to the nearest 0.01 ml. Treat the blanks in the same way as the sample.

Calculation

$$\%\ \text{total glycerol} = [(\text{B} - \text{S}) \times \text{N} \times 2.302 \times 900]/(\text{W} \times 50)$$

where

B	volume of 0.1 N sodium thiosulfate used for the blank, ml
S	volume of 0.1 N sodium thiosulfate used for the sample, ml
N	exact normality of 0.1 N sodium thiosulfate
W	mass of sample, g

Total citric acid

Information required

Total fatty acid

Principle: This method measures total fatty acids by extracting with diethyl ether.

Procedure
Weigh accurately 5 g of the sample into a 250-ml round-bottomed flask, add 50 ml of potassium hydroxide, ethanolic, TS, and reflux for 1 h on a boiling water bath.

Quantitatively transfer the contents of the saponification flask to a 1,000 ml separating funnel, using three 25 ml portions of water, and add 5 drops of methyl orange indicator solution.

Cautiously add 50% hydrochloric acid until the colour of solution changes to orange red.t. Add 1 ml of excess acid. Shake well to mix the contents and separate the fatty acids.

Cool to room temperature and extract the separated fatty acids with three 100 ml portions of diethyl ether. Combine the extracts, and wash with 50 ml portions of 10% sodium chloride solution until the washed sodium chloride solution becomes neutral.

Dry the ether solution with anhydrous sodium sulfate. Then evaporate off ether on a steam bath, leave additional 10 min on the steam bath, and weigh the residue. This is the weight of the total fatty acids.

Calculation:

$$\text{Total Fatty acids } \% = \frac{\text{mass of fatty acids g} \times 100}{\text{mass of sample g}}$$

ERYTHROSINE

Prepared at the 86th JECFA and published in FAO JECFA Monograph 22 (2018) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-0.1 mg/kg bw was established at the 37th JECFA (1991) and confirmed at the 86th JECFA (2018).

SYNONYMS INS No. 127, CI Food Red 14, CI (1975) No. 45430, Food Red No. 3, FD&C Red No. 3

DEFINITION Erythrosine consists of the disodium salt of 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride.

Erythrosine may be converted to the corresponding aluminium lake in which case only the requirements in the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names Disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate;

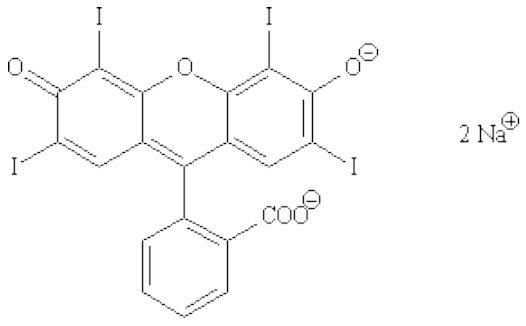
Disodium;2',4',5',7'-tetraiodo-3-oxospiro[2-benzofuran-1,9'-xanthene]-3',6'-diolate;

Disodium 2′,4′,5’,7′-tetraiodofluorescein monohydrate

C.A.S. number 16423-68-0

Chemical formula $C_{20}H_6I_4Na_2O_5 \cdot H_2O$

Structural formula



Formula weight 879.86

Assay Not less than 87% total colouring matters

DESCRIPTION	Red powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Soluble in water, slightly soluble in ethanol
Spectrophotometry (Vol. 4)	Maximum wavelength approximately 527 nm Determine the UV-visible absorption spectrum of the sample dissolved in water.
PURITY	
Loss on drying, chloride and sulfate as sodium salts (Vol. 4)	Not more than 13% Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under “Specific Methods, Food Colours”).
Inorganic iodides	Not more than 0.1% calculated as sodium iodide See description under TESTS
Water insoluble matter (Vol. 4)	Not more than 0.2%
Zinc (Vol. 4)	Not more than 50 mg/kg Determine using a method 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 (under “General Methods, Metallic Impurities”).
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method 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 (under “General Methods, Metallic Impurities”).
Subsidiary colouring matters	Not more than 4% (except fluorescein) See description under TESTS

Note: Do not allow the sample and standard solutions to be exposed to direct sunlight.

Fluorescein — Not more than 20 mg/kg

See description under TESTS

Organic compounds other than colouring matters — Triiodoresorcinol: Not more than 0.2%

2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid: Not more than 0.2%

See description under TESTS

Ether extractable matter (Vol. 4) — From a solution of pH not less than 7, not more than 0.2%

Hydrochloric acid-insoluble matter in Erythrosine Lake — Not more than 0.5%

See description under TESTS

TESTS

PURITY TESTS

Inorganic iodides — Weigh 1.0 g of the sample into a 100-ml beaker. Add 75 ml distilled water and a magnetic stirrer. Stir to dissolve. Immerse an iodide specific electrode and a reference electrode in the solution and use a suitable millivoltmeter to read the potential of the system in millivolts.

Add 0.001 M silver nitrate solution from a burette initially in 0.5 ml aliquots, reducing these to 0.1 ml as the end-point approaches as indicated by an increasing change in potential for each addition. After allowing time for the reading to stabilize, record the millivolt readings after each addition. Continue the titration until further additions make little change in the potential.

Plot the millivolt readings against the volume of silver nitrate solution added. The equivalence point is the volume corresponding to the maximum slope of the curve.

$$\text{The percentage of sodium iodide in the sample} = \text{Titre} \times 0.015\%$$

where

Titre	ml-equivalent of silver nitrate solution
0.015%	0.001 mol/l x 10^{-3} l/ml x 149.89 g sodium iodide/mol x 1 mol/equivalent x 1/1.0 g (sample weight) x 100.

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C8 (250 mm x 4.6 mm i.d., 5 µm particle size)
- Eluent A: 0.1 M ammonium acetate in water
- Eluent B: methanol
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 514 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	55	45
20	34	66
21.1	0	100
25.5	0	100
26.0	55	45
40.0	55	45

Reagents: HPLC grade

Standards:

- 2′,4′,5′-Triiodofluorescein (C.A.S. 56254-06-9) – synthesized material (see Appendix)
- 2′,4′,7′-Triiodofluorescein (C.A.S. 83498-90-2) – synthesized material (see Appendix)
- 4′,5′-Diiodofluorescein, disodium salt (C.A.S. 33239-19-9) – Alfa Aesar, Cat. No. A15626 or equivalent
- 2′-Monoiodofluorescein, disodium salt (C.A.S. 52010-85-2) – synthesized material (see Appendix)
- 4′-Monoiodofluorescein, disodium salt (C.A.S. 52010-86-3) – synthesized material (see Appendix)
- Erythrosine (C.A.S. 16423-68-0) – TCI, >95.0% disodium 2′,4′,5’,7′-tetraiodofluorescein, Cat. No. F0139 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 200±2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 514 nm. If Erythrosine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Erythrosine to the sum of all peaks.

Fluorescein

Determine fluorescein by the test for subsidiary colouring matters content except use the following conditions:

- Injection volume: 50 µl
- Detector: UV-visible/diode array at 492 nm

Standard: Fluorescein, disodium salt (C.A.S. 518-47-8) – TCI, Cat. No. F0096 or equivalent

Sample preparation:

Weigh accurately 2.00±0.05 g sample and dissolve in 10 ml of water.

Organic compounds other than colouring matters

Determine organic compounds other than colouring matters by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 (150 mm x 2.1 mm i.d., 5 µm particle size)
- Eluent A: 0.05 M sodium dihydrogen phosphate in 95/5 water/methanol, pH 4.0
- Eluent B: methanol
- Injection volume: 5 µl
- Column temperature: 27°
- Detector: UV-visible/diode array at 223 nm
- Flow rate: 0.5 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	95	5
3	95	5
5	80	20
13	35	65

15	0	100
25	0	100
27	95	5
37	95	5

Reagents: HPLC grade

Standards:

- 2,4,6-Triiodoresorcinol (C.A.S. 19403-92-0) – Alfa Chemistry, Cat. No. ACM19403920 or equivalent
- 2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid (C.A.S. 3480-21-5) – Wako, Cat. No. 043-32981 or equivalent

Prepare standard solutions as required. Dissolve the standards in methanol. Use an amber glass volumetric flask for 2,4,6-triiodoresorcinol and prepare the standard and calibration solutions immediately before use.

Sample preparation:

Weigh accurately 100±5 mg sample and dissolve in 10 ml of methanol.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 223 nm.

Hydrochloric acid-insoluble matter in Erythrosine Lake

Reagents

- Concentrated hydrochloric acid
- Hydrochloric acid, 0.5% v/v
- Dilute ammonium hydroxide solution (dilute 10 ml of 14.5 M ammonium hydroxide to 100 ml with water).

Procedure

Accurately weigh approximately 5 g of the lake into a 500-ml beaker. Add 250 ml water and 60 ml concentrated hydrochloric acid. Boil to dissolve the alumina while the Erythrosine converts to its "free acid" form, which is insoluble in acid. Filter through a tared No. 4 sintered glass crucible. Wash the crucible with a small amount of hot 0.5% hydrochloric acid and then with some hot distilled water. Remove the acid filtrate from the filter flask, replace the crucible, and wash with hot dilute ammonium hydroxide solution until the washings are colourless. Dry the crucible to constant weight at 135º. Express the residue as a percentage of the weight taken.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent.

Using water as the solvent:

absorptivity $(a) = 110\,l/(g \times cm)$

wavelength of maximum absorbance = 527 nm.

GLYCEROL ESTER OF WOOD ROSIN

Prepared at the 86th JECFA and published in FAO JECFA Monographs 22 (2018), superseding specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI of 0-25 mg/kg bw for glycerol ester of wood rosin was established at the 77th JECFA (2013).

SYNONYMS INS No. 445(iii)

DEFINITION Glycerol ester of wood rosin (GEWR) is a complex mixture of glycerol di- and tri- esters of resin acids from wood rosin, with a residual fraction of glycerol monoesters. In addition, neutrals (non-acidic saponifiable and unsaponifiable substances) and residual free resin acids are present. Wood rosin is obtained by the solvent extraction of aged pine stumps, followed by a liquid-liquid solvent refining process. Refined wood rosin is composed of approximately 90% resin acids and approximately 10% neutrals. The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main components are dehydroabietic and abietic acids. GEWR is produced by esterifying the resin acids with food grade glycerol. The product is then purified by steam stripping or by direct countercurrent steam distillation.

These specifications do not cover substances derived from gum rosin, an exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing.

C.A.S. number 8050-30-4

DESCRIPTION Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES Emulsifier, density adjustment agent (flavouring oils in beverages), stabilizer, plasticizer (in chewing gum bases).

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in acetone

Infrared absorption (Vol. 4) The infrared spectrum of a thin film of the sample (potassium bromide disc) corresponds with the typical infrared spectrum below

Sulfur test	Negative Weigh 40-50 mg of sample into a test tube and add 1- 2 drops of a 20% (w/v) solution of sodium formate. Place a strip of lead acetate test paper over the mouth of the test tube. Heat the tube until fumes are formed that contact the test paper. Continue heating for 2-5 min. The formation of a black spot of lead sulfide indicates the presence of sulfur-containing compounds. (Detection Limit: 50 mg/kg sulfur)
Gas chromatography of resin acids and glycerol	Passes test See description under TESTS

PURITY

Specific gravity (Vol. 4)	d (20, 25): Not less than 0.935 (50% solution in d-limonene)
Ring and ball softening point (Vol. 4)	Not less than 82° (see “Specific Methods, Glycerol Esters of Rosins”)
Acid value (Vol. 4)	Between 3 and 9 (see “Specific Methods, Fats, Oils, and Hydrocarbons”)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using method 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 (under “General Methods, Metallic Impurities”).

TESTS

IDENTIFICATION TESTS

Gas chromatography of resin acids	The ester groups in the glycerol esters of wood rosin are reduced with a metal hydride to form a mixture of corresponding resin alcohols and glycerol which are analyzed by gas chromatography (Vol. 4). The characteristic chromatogram shows predominant peaks for abietic and dehydroabietic alcohols. Apparatus - Gas Chromatograph equipped with a flame ionization detector. - Centrifuge: table top, capable of achieving 3200 rpm

Standards and reagents

- Internal Standard (1,4-Butanediol: >99%
- Toluene
- Sodium Vitride Reagent [(Sodium bis(2-methoxyethoxy) aluminium dihydride], 70% in toluene:(~ 3.5 mol/l)

Sodium Vitride solution:

Pipet 10.0 ml of sodium vitride reagent into a 100 ml volumetric flask dilute to volume with toluene and mix thoroughly.

Hydrolysis solution:

Slowly add 50 ml of concentrated sulfuric acid, reagent grade, to 200 ml distilled water while stirring in an ice bath. Cool to room temperature.

Procedure

Sample preparation

Weigh 250-300 mg sample into a 25 ml Erlenmeyer flask containing a Teflon coated stirrer bar. Pipet 5.0 ml toluene into the flask and stir until sample is dissolved. Pipet 5.0 ml of sodium vitiride solution into the flask, stopper the flask and stir for 30 min. While stirring, pipet 3.0 ml of hydrolysis solution into the flask. Continue stirring for 3 min. Transfer contents of flask to centrifuge tube (15 ml), stopper, and shake vigorously. Vent and centrifuge at 2800-3200 rpm for 5 min. Inject 0.5 µl of the toluene layer into the gas chromatograph operating under the following conditions and record the chromatogram. Compare with the chromatogram shown below to verify the approximate retention order of the resin alcohols.

Chromatographic conditions

- Column: DB-1 methyl silicone (bonded and crosslinked) wide-bore capillary (15 m x 0.53 mm i.d.,1.5 µm).
- Injector: Flash vaporization injector
- Flow rates: Carrier Gas (He): 30 ml/min at 63 psi, Hydrogen: 30 ml/min and
- Air: 240 ml/min
- Temperatures: Column: Isothermal, 190º; Injector: 250º , and Detector: 250º

Gas chromatography of glycerol

Standards and reagents

- Glycerol: >99%
- 1,4-Butanediol (Internal standard): >99%

Internal Standard Solution:

Weigh 0.1 g of 1,4-butanediol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.

Glycerol solution:

Weigh 0.1 g of 1,4-butanediol and 0.1 g glycerol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly

Phenolphthalein Solution: 1% in ethanol.

Sodium Hydroxide Solution:

Dissolve 16 g of reagent grade NaOH in 70-80 ml of distilled water and cool to room temperature. Dilute to 100 ml with distilled water and mix thoroughly. Store in a polyethylene bottle.

Procedure

Sample preparation

Proceed as in the sample preparation for the analysis of resin acids until the centrifugation step. Using a pipet or syringe, remove the toluene layer and part of the aqueous layer leaving approximately 2 ml of the aqueous layer in the centrifuge tube. Add 1 drop of phenolphthalein solution to the remaining aqueous layer in the centrifuge, and neutralize with the sodium hydroxide solution (aluminium salts will precipitate). Pipet 5 ml of the internal standard solution into the tube, dilute to 15 ml with distilled water, stopper, shake, and then centrifuge at 2800-3200 rpm for 5 min. Inject 1 µl of the clear supernatant liquid into the gas chromatograph operating under the following conditions and record the chromatogram. Inject 1 µl of the glycerol solution and record the chromatogram. Measure the retention times of any observed peaks relative to 1,4-butanediol. Compare retention times to that of glycerol standard.

Chromatographic conditions

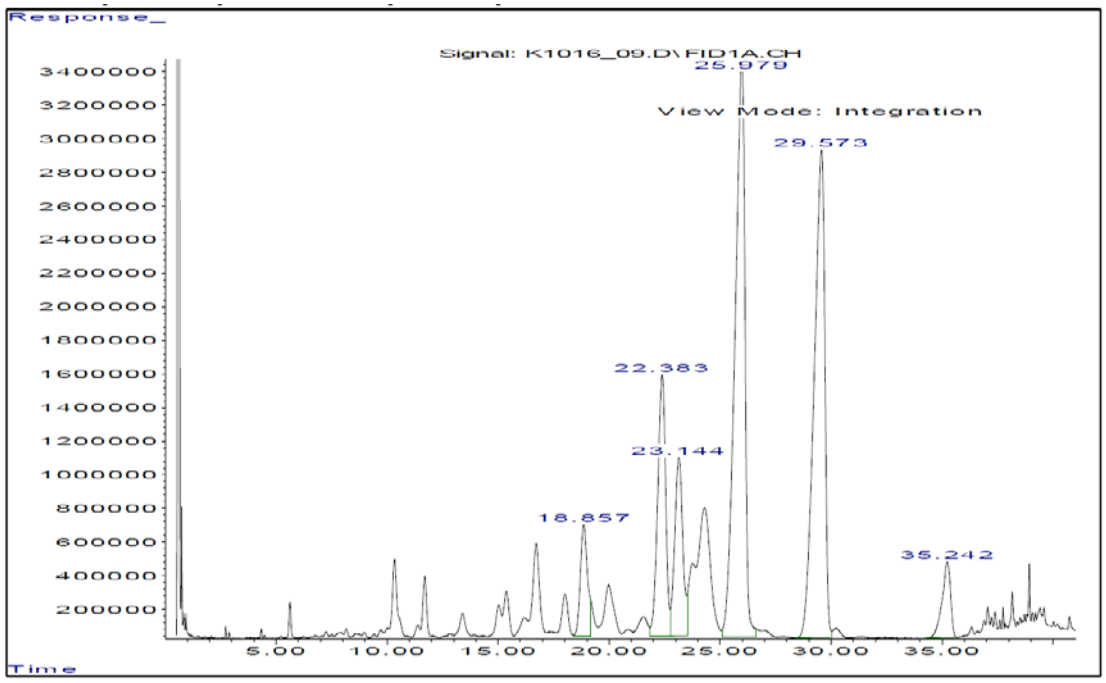
- Column: DB-WAX polyethyleneglycol (bonded and cross-linked), wide bore capillary (15 m x 0.53 mm i.d., 1.0 µm)
- Flow rates: Carrier Gas (He): 30 ml/min at 60 psi, Hydrogen: 30 ml/min and
- Air: 240 ml/min
- Temperatures: Column: Programmed, 120 to 200º at 6º /min; Injector: 250º,
- and Detector: 250º

Gas chromatography of resin acids in GEWR (determined as alcohols)

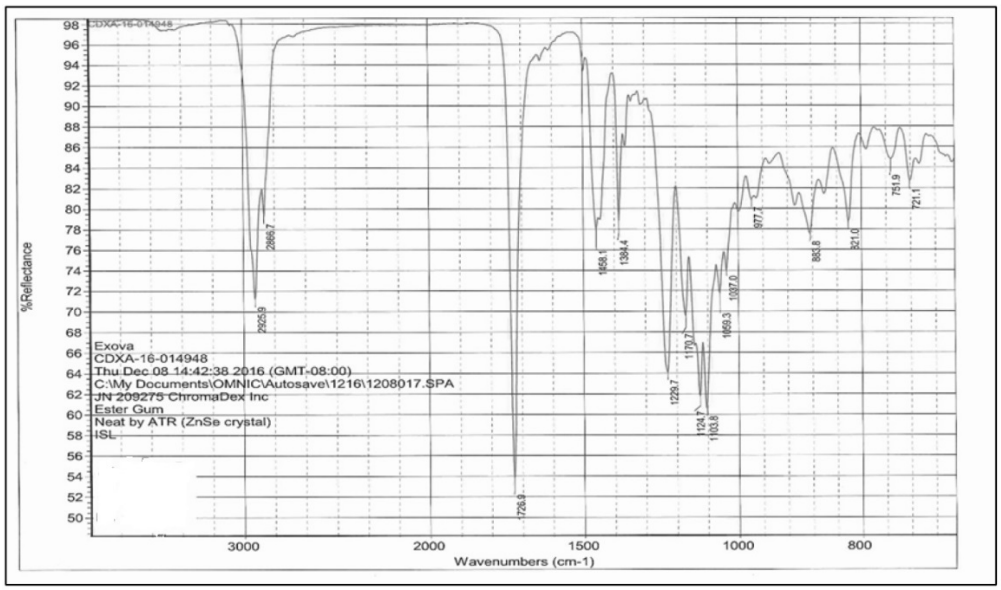
Typical GC-FID chromatogram of a GEWR sample. Retention times correspond to pimaric (18.8 min), isopimaric (22.4 min), palustric (23.1 min), dehydroabietic (25.9 min), abietic (29.6 min), and neoabietic (35.2 min) alcohols. This is a product

derived from a plant-based source which can demonstrate significant variability and relative intensities.

Gas chromatogram



FTIR Spectrum of Glycerol esters of wood rosin



INDIGOTINE

Prepared at the 86th JECFA and published in JECFA Monograph 22 (2018) superseding specifications prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-5 mg/kg bw was established at the 18th JECFA (1974) and confirmed at the 86th JECFA (2018).

SYNONYMS INS No. 132, CI Food Blue 1, CI (1975) No. 73015, Indigo Carmine, Food Blue No. 2, FD&C Blue No. 2

DEFINITION Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate and disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Indigotine is manufactured by heating indigo in the presence of sulfuric acid. The indigo (or indigo paste) is manufactured by the fusion of N-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. It is isolated and subjected to purification procedures prior to sulfonation.

Indigotine may be converted to the corresponding aluminium lake in which case only the requirements in the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names Disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate

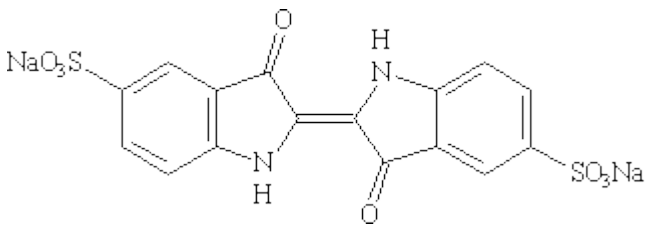
Disodium (2E)-3-oxo-2-(3-oxo-5-sulfonato-2,3-dihydro-1H-indol-2-ylidene)-2,3-dihydro-1H-indole-5-sulfonate

Disodium;(2E)-3-oxo-2-(3-oxo-5-sulfonato-1H-indol-2-ylidene)-1H-indole-5-sulfonate

C.A.S. number 860-22-0 (5,5' isomer)

Chemical formula $C_{16}H_8N_2Na_2O_8S_2$

Structural formula



Formula weight 466.36

Assay	Not less than 85% total colouring matters Not more than 18% of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate
DESCRIPTION	Blue powder or granules
FUNCTIONAL USES	Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water, sparingly soluble in ethanol
Spectrophotometry (Vol. 4)	Maximum wavelength approximately 610 nm Determine the UV-visible absorption spectrum of the sample dissolved in water.

PURITY

Loss on drying, chloride and sulfate as sodium salts (Vol. 4)	Not more than 15% Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under “Specific Methods, Food Colours”).
Water insoluble matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method 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 (under “General Methods, Metallic Impurities”).
Subsidiary colouring matters	Not more than 18% disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (isomeric subsidiary colouring matter) Not more than 1% other subsidiary colouring matters See description under TESTS
Organic compounds other	Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-

than colouring matters	sulfoanthranilic acid, and anthranilic acid See description under TESTS
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.2% Weigh accurately about 2 g sample instead of the 5 g stated in the general methods

TESTS

PURITY TESTS

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 (250 mm x 4 mm i.d., 5 µm particle size)
- Eluent A: 0.2 M ammonium acetate in water
- Eluent B: acetonitrile
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 610 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
20.0	40	60
30.0	40	60
32.0	100	0
40.0	100	0

Reagents: HPLC grade

Standards:

- Indigotine disodium salt (5,7′ isomer) (isomeric subsidiary colour) (C.A.S. 27414-68-2) – Angene Chemical, Cat. No. AGN-PC-0R372R or equivalent
- Sodium indigo sulfonate monosodium salt (monosulfonated subsidiary colour) (C.A.S. 27414-69-3) – Atomax Chemicals Co., Ltd., Cat No. AM27414693 or equivalent

- Trisodium indigo-5,5′,7′-trisulfonate
- (trisulfonated subsidiary colour), potassium salt (C.A.S. 67627-18-3) – Sigma-Aldrich, Cat No. 234087 or equivalent
- Indigotine (C.A.S. No. 860-22-0) – TCI, Cat. No. F0148 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:
Weigh accurately 100±2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution. Analyze immediately after preparation.

Calculations:
Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 610 nm. If Indigotine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Indigotine to the sum of all peaks.

Organic compounds other than colouring matters

Determine organic compounds other than colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: Luna C18 (250 mm x 4.6 mm i.d., 5 µm particle size) or equivalent
- Eluent A: 0.1% trifluoroacetic acid in water
- Eluent B: acetonitrile
- Injection volume: 20 µl
- Column temperature: 25°
- Detector: UV-visible/diode array at 244 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
15	80	20
20	0	100
22	0	100
22.1	100	0
32	100	0

Reagents: HPLC grade

Standards:

- Isatin-5-sulfonic acid sodium salt dihydrate (C.A.S. 207399-16-4) – Sigma-Aldrich Cat. No. 58245 or equivalent
- 5-Sulfoanthranilic acid (2-amino-5-sulfobenzoic acid) (C.A.S. 3577-63-7) – TCI Cat No. S0802 or equivalent
- Anthranilic acid (C.A.S. 118-92-3) – Sigma-Aldrich Cat No. A89855 or equivalent

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 100±2 mg sample and dissolve in 100 ml of water. Analyse immediately after preparation.

Calculations:

Construct the relevant standard curves. Integrate the chromatogram peaks obtained at 244 nm.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under "Specific Methods, Food Colours") and an appropriate solvent. Analyse immediately after preparation.

Using water as the solvent:

absorptivity $(a) = 48.0\ l/(g \times cm)$
wavelength of maximum absorbance = 610 nm.

Determine isomer content by HPLC using the test for subsidiary colouring matters.

LUTEIN FROM TAGETES ERECTA

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications prepared at the 63rd JECFA (2004) and published in FNP52 Add 12 (2004). A group ADI of "not specified" was established for Tagetes extract, Lutein from Tagetes erecta, Lutein esters from Tagetes erecta, Zeaxanthin (synthetic), and meso-zeaxanthin at the 86th JECFA (2018) superseding the group ADI of 0 - 2 mg/kg bw for lutein from T. erecta L. and synthetic zeaxanthin established at the 63rd JECFA (2004).

SYNONYMS INS No. 161b(i), Vegetable lutein; vegetable luteol; Bo-Xan, luteine

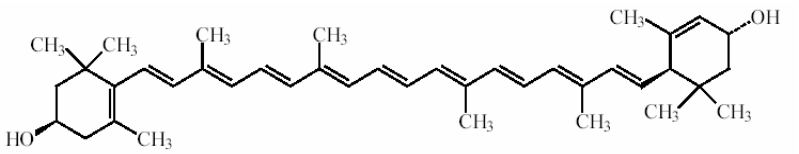
DEFINITION Lutein from *Tagetes erecta* is a purified extract of xanthophylls obtained from oleoresin in marigold. The oleoresin is prepared from hexane extracts of *Tagetes erecta* L. flowers, saponified with potassium hydroxide in either methanol or propylene glycol. The resulting reaction mixture is diluted with water and dried. The crystalline product contains lutein along with minor components that include other carotenoids and waxes.

Chemical names 3R,3'R,6'R-β,ε-carotene-3,3'-diol; all-*trans*-lutein; 4',5'-didehydro-5',6'-dihydro-beta,beta-carotene-3,3'-diol

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

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

Structural formula



Formula weight 568.88

Assay Not less than 80% total carotenoids, not less than 70% lutein

DESCRIPTION A free-flowing, orange-red powder

FUNCTIONAL USES Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, soluble in hexane
Spectrophotometry (Vol. 4)	A 2 mg/l solution in acetone shows maximum absorbance at approximately 446 nm.
Test for carotenoids (Vol. 4)	The colour of 2 ml of a 2 – 4 mg/l solution of the sample in acetone immediately disappears after successive addition of about 0.5 ml of 5% sodium nitrite and about 0.5 ml of 0.5 M sulfuric acid.

PURITY

Moisture (Vol. 4)	Not more than 1.0%
Ash (Vol. 4)	Not more than 1.0%
Zeaxanthin	Not more than 9.0% See description under METHOD OF ASSAY
Lead (Vol. 4)	Not more than 3 mg/kg. Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Hexane (Vol. 4)	Not more than 50 mg/kg
Methanol (Vol. 4)	Not more than 10 mg/kg
Propylene glycol	Not more than 1000 mg/kg See description under TESTS
Waxes	Not more than 14.0% See description under TESTS

TESTS

PURITY TESTS

Propylene glycol

Determine by gas chromatography (Vol. 4) under the following conditions.

Internal standard solution

Prepare a 500 µg/ml solution of ethylene glycol in tetrahydrofuran.

Standard solutions

Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 µg/ml of propylene glycol and 5 µg/ml of ethylene glycol in tetrahydrofuran.

Chromatography conditions

- Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
- Carrier gas: Helium
- Flow rate: 1.5 ml/min (Constant flow)
- Detector: FID
- Temperatures: injection port: 230°
- Column Temperature: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
- Detector Temperature: 270°

The retention times of ethylene glycol and propylene glycol derivatives under the above conditions are approx. 7.6 min and
7.8 min, respectively.

Procedure

Weigh accurately 1 g of the sample into a 10-ml volumetric flask, and add 100 µl of the internal standard solution. Dissolve and make up to volume with tetrahydrofuran. Take 0.5 ml of the sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS). After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 µl of this centrifugal supernatant into the chromatograph.

Standard curve

Prepare following the same procedure using 0.5 ml of the standard solutions in place of the sample solution.

Calculate the concentration of propylene glycol in mg/kg (C_{PG}) from:

$$\mathrm{CPG\ (mg/kg)} = \mathrm{C} \times 10 / \mathrm{W}$$

where
C is polyethylene glycol concentration determined (µg/ml); and
W is weight of sample (g)

Waxes

Determine by gas chromatography (Vol. 4) using the following conditions:

- GC column DB-5 (30 m x 0.25 mm ID with a 0.25 µm film thickness) or equivalent.
- GC injector temperature: 280°
- FID temperature: 350°
- GC temperature program: 50° (2 min) 13°/min to 340° and hold for 8 min
- Carrier gas (Helium) flow rate: 1.0 ml/min
- Injection mode: splitless
- Injection volume: 1.0 µl

Standards:

- Hydrocarbons mixed standard: C25 to C46
- Internal standard: Hexatriancontane (C36)

Standard solutions:

Prepare standard solutions by addition of hydrocarbon standards to methylene chloride to get hydrocarbon concentrations of 2.0, 5.0, 10, 25, 50, mg/l respectively. Add required quantity of hexatriancontane internal standard to get a final concentration 50 mg/l in all standard solutions.

Sample Preparation

Accurately weigh 100 mg of sample into a centrifuge tube and dissolve in exactly 20 ml of methylene chloride. Sonication or vortex mixing may be required to completely dissolve the product. Centrifuge sample at 2500 rpm for 5 min, if the sample appears turbid. Add 1.6 ml of methylene chloride and 20 µl of (5000 mg/l) hexatriancontane solution (to a final concentration of 50 mg/l) into 2 ml volumetric flask. Transfer 40 µl of sample solution and dilute with methylene chloride to the 2 ml. Transfer the solution into a 2 ml autosampler vial.

Analysis

Inject 1.0 µl of each of the standards solutions. Record the peak areas. Construct standard curves using the peak ratios of each hydrocarbon to the internal standard against the concentration of the hydrocarbon. Inject 1.0 µl of the sample solution and determine individual wax in the sample (mg/l) from the respective standard curve.
Add the concentration of individual waxes to get the total wax concentration in the sample solution (mg/l)

Calculation:

$$\text{Waxes \% w/w} = \frac{\text{C (mg/l)} \times 2\text{ ml} \times 20\text{ ml} \times 100}{1000\text{ (ml/l)} \times \text{W (mg)} \times 0.04\text{ ml}}$$
$$= (100 \times \text{C})/\text{W}$$

Where:
C is the total concentration of waxes, mg/l in the sample
W is the weight of sample, mg

METHOD OF ASSAY

Determine the total carotenoid content and the content of lutein and zeaxanthin by UV spectrophotometry and HPLC using the following conditions:

Reagents:

- Hexane (HPLC grade)
- Ethyl acetate (HPLC grade)
- Acetone
- Dehydrated ethyl alcohol (absolute alcohol)
- Toluene
- Solvent Mixture: (10:6:7:7 hexane:dehydrated ethyl alcohol:acetone:toluene, v/v/v/v)

System Suitability Solution for HPLC:

150 µg/ml of lutein standard in solvent mixture (use USP Lutein RS available from U. S. Pharmacopeia, or equivalent standard)

Apparatus

UV/Vis spectrophotometer; 1-cm cuvettes
HPLC system with suitable diode array detector, autosampler, column oven, signal processor and degasser.
Analytical column: 3 µm silica, 4.6 mm x 250 mm

Instrument Conditions

- Temperature: ambient
- Mobile Phase: 70:30 (v:v) hexane/ethyl acetate (isocratic elution)
- Flow Rate: 1.5 ml/min
- Injection: 10 µl
- Detection: UV/Vis 446 nm
- Run Time: approximately 40 min

Concentrated Sample Preparation

For the UV/Vis spectrophotometry weigh sample (30 mg) into a glass weighing funnel. Using the solvent mixture, wash crystals into a 100 ml volumetric flask, dilute to the mark with the solvent mixture and stir for 10 min.

Sample Preparation

Pipette 1 ml of concentrated sample preparation into a

100 ml volumetric flask. Dilute up to the mark with dehydrated ethyl alcohol, mix by inversion for 20 seconds. Read samples in a spectrophotometer at 446 nm using dehydrated ethyl alcohol as the blank.

For HPLC, evaporate 1 ml of the concentrated sample preparation to dryness using a stream of nitrogen, dissolve solids in 1 ml 70:30 hexane:ethyl acetate, and add 0.5 ml to HPLC vials. Analyze this sample and the system suitability solution for HPLC using the HPLC conditions above.

Results

Compare the results of the chromatogram from the system suitability solution for HPLC to identify the lutein and zeaxanthin peaks at a resolution of not less than 3.

Calculation

Using the results obtained from the UV/Vis spectrophotometry calculate the % Total carotenoids

$$\%\ \text{Total carotenoids} = \frac{\text{Absorbance at } 446\ \text{nm} \times 10000 \times 100}{\text{sample weight in g} \times 2550}$$

Note: The factors 10000 and 2550 are the dilution factor and extinction value for a 1% solution, respectively.

Using the chromatogram of the sample, calculate the concentration of lutein and zeaxanthin.

$$Lutein\ (\%) = \%\ Total\ carotenoids \times \frac{Peak\ Area\ LUTEIN}{Peak\ Area\ TOTAL}$$

$$Zeaxanthin\ (\%) = \%\ Total\ carotenoids \times \frac{Peak\ Area\ ZEAXANTHIN}{Peak\ Area\ TOTAL}$$

MODIFIED STARCHES

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications included in 16 individual specification monographs prepared at the 82nd JECFA (2016), published in FAO JECFA Monographs 19 (2016).

MODULAR MONOGRAPH consisting of "GENERAL SPECIFICATIONS"[(a)] that contains common specifications to all modified starches(INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and 8 ANNEXES that contain specifications related to the chemical treatments of native starches:

- ANNEX 1[(a)] – Fragmentation.
- ANNEX 2[(a)] – Bleaching.
- ANNEX 3[(a)] – Esterification and/or crosslinking with phosphorous containing compounds.
- ANNEX 4[(a)] – Acetylation.
- ANNEX 5[(a)] – Oxidation.
- ANNEX 6[(a)] – Esterification with octenyl succinic anhydride.
- ANNEX 7[(a)] – Etherification with propylene epoxide.
- ANNEX 8[(a)] – Esterification and crosslinking with adipic anhydride.

The General specifications are applicable to the following modified starches, each of which should additionally fulfil the specifications of the ANNEXES as follows:

Modified Starch	**INS**	**Annex**	**ADI**	**STATUS**
Dextrin roasted starch	1400	1	N.S.[(1)]	T[(3)]
Acid treated starch	1401	1	N.S.[(1)]	T[(3)]
Alkaline treated starch	1402	1	N.S.[(1)]	T[(3)]
Bleached starch	1403	2	N.S.[(1)]	T[(3)]
Oxidized starch	1404	5	N.S.[(1)]	T[(3)]
Enzyme-treated starch	1405	1	N.S.[(1)]	T[(3)]
Monostarch phosphate	1410	3	N.S.[(1)]	T[(3)]
Distarch phosphate	1412	3	N.S.[(1)]	T[(3)]
Phosphated distarch phosphate	1413	3	N.S.[(1)]	T[(3)]
Acetylated distarch phosphate	1414	3, 4	N.S.[(1)]	T[(3)]

Starch acetate	1420	4	N.S.[1]	T[3]
Acetylated distarch adipate	1422	4, 8	N.S.[1]	T[3]
Hydroxypropyl starch	1440	7	N.S.[1]	T[3]
Hydroxypropyl distarch phosphate	1442	3, 7	N.S.[1]	T[3]
Starch sodium octenylsuccinate	1450	6	N.S.[1]	T[3]
Acetylated oxidized starch	1451	4, 5	N.S.[2]	T[3]

Should any of the modified starches be subjected to additional chemical treatment, the appropriate specifications outlined in the respective ANNEX should be met. Consequently, for all fragmented and/or bleached starches the specifications of ANNEXES 1 and/or 2 respectively should be met.

[a]Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications included in specification monographs prepared at the 82nd JECFA (2016), published in FAO JECFA Monographs 19(2016).

[1]An ADI "not specified" was established at the 26th JECFA (1982).
[2]An ADI "not specified" was established at the 57th JECFA (2001).
[3] T: TENTATIVE

Summary Table

GENERAL REQUIREMENTS

	IDENTIFICATION			**PURITY**				
Solubility	**Microscopy**	**Iodine Stain**	**Copper Reduction**	**pH**	**Loss on Drying**	**Lead**	**Microbiological Criteria**	**Sulfur dioxide**
Insoluble in cold water	Granular structure typical of the starch source	Colour from dark blue to red after addition of tri-iodide	Red precipitate after addition of hot alkaline cupric tartrate to a test sample refluxed under acidic condition	**3.0 -9.0**	Cereal starch ≤15.0%; Potato starch: ≤21.0%; Other starches: ≤18.0%	≤0.2mg/kg d.w. Pb (≤0.1 mg/kg) for OSA for infant formula	Aerobic Plate Count: ≤1000 CFU/g; Yeasts and molds: ≤1000 CFU/g; Total Coliforms: ≤10 cfu/g; Information required.	≤50 mg/kg d.w. for modified cereal starches; ≤10 mg/kg d.w. for other modified starches

SPECIFIC REQUIREMENTS

Modified Starch	Annex	**IDENTIFICATION**	**PURITY**
Dextrin roasted (INS 1400)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Acid treated (INS 1401)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Alkaline treated starch (INS 1402)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Bleached (INS 1403)	2	No additional	Carboxyl groups (≤0.1% d.w.);Residual oxidising substances (Information Required)
Oxidized (INS 1404)	5	hypochlorite oxidized starch	Carboxyl groups (≤1.3% d.w.);Residual hypochlorite (Information Required)
Enzyme-treated (INS1405)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Monostarch phosphate (INS 1410)	3	Information required	Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.wfor other starches)
Distarch phosphate (INS 1412)	3	Information required	Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)
Phosphated distarch phosphate (INS 1413)	3	Information required	Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)
Acetylated distarch phosphate (INS 1414)	3, 4	Acetyl group; Ester group; Information required	Phosphate (≤0.14% d.w. for potato or wheat starches; ≤0.04% d.w. for other starches) Acetyl groups (≤2.5%d.w.); Ester groups (≤0.5%d.w.)
Starch acetate (INS 1420)	4	Acetyl group; Ester group	Acetyl groups (≤2.5%d.w.); Ester groups (≤0.5%d.w.)
Acetylated distarch adipate (INS 1422)	4, 8	Acetyl group; Ester group; Information required	Acetyl groups (≤2.5%d.w.); Vinyl acetate (≤0.1 mg/kg); Ester groups (≤0.5%d.w.) Adipate groups (≤0.135%d.w.); Residual free adipic acid (Information Required)
Hydroxypropyl starch (INS 1440)	7	Hydroxypropyl ether groups	Hydroxypropyl groups (≤7.0% d.w.); Propylene chlorohydrins (≤1 mg/kg d.w.)
Hydroxypropyl distarch phosphate (INS 1442)	3, 7	Hydroxypropyl ether groups; Information required	Phosphate (≤0.14% d.w. for potato or wheat starches; ≤0.04% d.w. for other starches) Hydroxypropyl groups (≤7.0% d.w.); Propylene chlorohydrins (≤1 mg/kg d.w.)
Starch sodium octenylsuccinate (INS 1450)	6	No additional	Octenylsuccinyl groups (≤3% d.w.); Residual free octenylsuccinic acid (≤0.3%d.w.);
Acetylated oxidized starch (INS 1451)	4, 5	Acetyl group	Acetyl groups (≤2.5%d.w.); Vinyl acetate (≤0.1 mg/kg); Ester groups (≤0.5%d.w.) Carboxyl groups (≤1.3% d.w.);Residual hypochlorite (Information Required)

GENERAL SPECIFICATIONS FOR MODIFIED STARCHES

(VERSION 2018 - TENTATIVE)

Information required:

- *Suitable microbiological acceptance criteria and supporting data*

DEFINITION

Starch consists mainly of amylose and amylopectin. Amylose is a linear molecule of α-D-glucopyranosyl units linked by (1-4)-α-linkages. Amylopectin is a highly-branched polymer of α-D-glucopyranosyl units linked by (1-4)-α-linkages and by(1-6)-α-linkages that constitute the branch points. Each glucose unit possesses a maximum of three hydroxyls that can undergo chemical substitution.

Native starches can be physically (pre-gelatinized starches) and/or chemically modified for improved functionality. The most common sources of native starch used in these modifications are various roots, tubers, cereals and legumes. Modified starches are used in applications requiring special properties not attainable by native starches.

Chemical modifications of native starches are often performed, in an aqueous suspension under controlled conditions of pH, time and temperature, unless otherwise indicated in the description of the respective annex. After sufficient reaction time, the modified starch is recovered by filtration or centrifugation, washed with water, dried and packaged. The relevant modification reactions can be, separately or in combination, fragmentations (hydrolysis, oxidation, enzymatic), bleaching, oxidation, esterification, etherification or phosphorylation of one or more of the hydroxyl groups of the α-D-glucopyranosyl units or crosslinking using polyfunctional agents.

See the appropriate Annex or Annexes for the treatment that is applicable to individual modified starch products.

C.A.S numbers

See ANNEXES

DESCRIPTION

White or nearly white powder or granules or (if pre-gelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES

Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in cold water (if not pre-gelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.
Microscopy	Passes test See description under TESTS
Iodine stain	Passes test See description under TESTS
Copper reduction	Passes test See description under TESTS

PURITY

General Requirements:

pH	3.0 – 9.0 See description under TESTS
Loss on drying (Vol 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% Conditions: 120°, 4 h, vacuum not exceeding 100 mm Hg
Lead (Vol. 4)	Not more than 0.2 mg/kg on the dried basis Not more than 0.1 mg/kg on the dried basis for Starch sodium octenylsuccinate (INS 1450) for use in infant formula and formula for special medical purposes intended for infants(see Annex 6) Determine using a method 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”).

Microbiological Criteria (Vol 4)	Aerobic plate count: Not more than 1000 CFU/g Yeasts and moulds: Not more than 1000 CFU/g Total coliforms: Not more than 10 CFU/g Information required
Sulphur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches

TESTS

IDENTIFICATION TESTS

Microscopy	Each modified starch, which has not been pre-gelatinized, retains its granular structure and can be identified as a starch by microscopic observation. The typical polarization cross is observed when sample is examined with a polarizing microscope, in polarized light under crossed Nicol prisms. Corn starch: Polygonal, rounded or spherical granules up to 35 µm diameter having a circular or several-rayed central cleft. Potato starch: Irregular shaped, ovoid, pear-shaped granules (30-100 µm diameter, occasionally >100 µm); both, the ovoid, the pear-shaped granules and the rounded granules have an eccentric hilum. All granules show clearly visible concentric striations. Tapioca starch: Spherical granules with one truncated side (5-35 µm diameter) usually having a circular or several-rayed central cleft. Wheat starch: large and small granules (10-60 µm diameter). The central hilum and striations are visible and barely visible.
Iodine stain	Add a few drops of 0.1 N potassium triiodide to an aqueous suspension of the sample. The modified starch stains with iodine in the same way as native starches. The colour can range from dark blue to red.
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask; add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water; mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced.

PURITY TESTS

pH (Vol. 4)	Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinised starches, 3 g should be suspended in 97 ml of water).

ANNEX 1: ADDITIONAL SPECIFICATIONS FOR STARCHES MODIFIED BY FRAGMENTATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- *A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes*

APPLIES TO

Dextrin roasted starch (INS No. 1400)

Acid treated starch (INS No. 1401)

Alkaline treated starch (INS No. 1402)

Enzyme-treated starch (INS No. 1405)

All modified starches that are fragmented

SYNONYMS

Modified starch by fragmentation, converted starch, hydrolysed starch.

TREATMENT

The fragmentation of native starch results in products containing polymers with a lower average molecular weight and reduced viscosity. The manufacturing details for the various modified starches by fragmentation in this monograph are described as below:

- *Dextrin roasted starch, INS. 1400*: is manufactured by dry heating or roasting of native starch with hydrochloric acid or ortho-phosphoric acid in heated and/or agitated vessels. The final dextrin roasted starch is obtained by drying.
- *Acid treated starch, INS. 1401* is obtained by treating a slurry or a suspension of native food starch with dilute hydrochloric acid, ortho-phosphoric acid, or sulphuric acid.
- *Alkaline treated starch, INS. 1402* is obtained by treating a suspended solution of native food starches with sodium hydroxide or potassium hydroxide.
- *Enzyme-treated starch, INS 1405* is obtained by treating a suspension of native food starch with one or more food-grade amyolytic-enzymes (e.g., α-amylase (E.C. 3.2.1.1), β-amylase (3.2.1.2), glucoamylase (3.2.1.3), isoamylase (3.2.1.68), pullulanase (E.C. 3.2.1.41)).

The properties of the modified starches by fragmentation vary depending on the source of native starch, reaction conditions (pH, reaction time, reaction temperature, fragmenting reagent etc.) The alteration of native starch allows for applications that require reduced viscosity in hot solutions and/or typically utilise high levels of modified starches.

C.A.S number

9004-53-9 (Dextrins)

65996-63-6 (Acid-hydrolysed starch)

68909-37-5 (Acid-hydrolysed amylopectin)

9005-84-9 (Starch soluble)

65996-64-7 (Enzyme-hydrolysed starch)

1001439-91-3 (Enzyme-treated amylopectin).

CHARACTERISTICS

IDENTITY

Dispersion identity — Information required.

Reducing sugars — Information required

TESTS

IDENTIFICATION TESTS

Dispersion test — Information required

Reducing sugars — Information required

ANNEX 2: ADDITIONAL SPECIFICATIONS FOR BLEACHED STARCHES

(VERSION 2018 - TENTATIVE)

Information is required on:

- *Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s).*

APPLIES TO	Bleached starch INS No. 1403 All modified starches that are bleached
TREATMENT	Peracetic acid and/or hydrogen peroxide, or sodium hypochlorite, sodium chlorite, sulfur dioxide, alternative permitted forms of sulphites, potassium permanganate, or ammonium persulfate Bleaching is performed to improve physical attributes such as colour due to oxidation of traces of pigments such as carotenoids and xanthophylls. The change is essentially in the colour only. Residual reagents are either removed or limited to technically unavoidable levels.
C.A.S number	977075-42-5 and all other modified starches submitted to bleaching

CHARACTERISTICS

PURITY

Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method 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”).
Residual oxidising substances	Information required
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis applying the correction for phosphate content as outlined in Note 6 of the method for starches esterified with phosphorus containing compounds.

ANNEX 3: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED AND/OR CROSSLINKED WITH PHOSPHORUS CONTAINING COMPOUNDS

(VERSION 2018 - TENTATIVE)

Information required on: A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches.

APPLIES TO

Monostarch phosphate (INS No. 1410)

Distarch phosphate (INS No. 1412)

Phosphated distarch phosphate (INS No. 1413)

Acetylated distarch phosphate (INS No. 1414)

Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

The phosphorus containing compounds ortho-phosphoric acid, sodium or potassium ortho-phosphate and sodium tripolyphosphate, can be used for esterification and the sodium trimetaphosphate or phosphorus oxychloride for crosslinking.

- *Monostarch phosphate (INS 1410)* is obtained by esterification/crosslinking of unmodified food starch with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate
- *Distarch phosphate (INS 1412)* is obtained by crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride
- *Phosphated distarch phosphate (INS 1413)* is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate
- *Acetylated distarch phosphate (NS 1414)* is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate
- *Hydroxypropyl distarch phosphate (INS1442)* is obtained by esterification of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide

Phosphorylation results in partial substitution of the 2, 3- or 6- position of the anhydro glucose unit unless the 6-position is occupied for branching. In the case of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be

represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

C.A.S numbers

Monostarch phosphate (INS 1410)
11120-02-8(Modified starch)
63055-37-8 (Modified amylopectin)

Distarch phosphate (INS No. 1412)
55963-33-2(Modified starch)
63055-37-8 (Modified amylopectin)

Phosphated distarch phosphate (INS No. 1413)
11120-02-8(Modified starch)
63055-37-8 (Modified amylopectin)

Acetylated distarch phosphate (INS No. 1414)
9067-33-8(Modified starch)
68130-14-3(Modified starch)
113894-91-0 (Modified amylopectin)

Hydroxypropyl distarch phosphate (INS No. 1442)
53124-00-8(Modified starch)
113894-92-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

Phosphate (calculated as phosphorus) (Vol. 4)

For monostarch phosphate (INS No. 1410), distarch phosphate (INS No. 1412), and phosphate distarch phosphate (INS No. 1413)

Not more than 0.5% on the dried basis for potato or wheat starches

Not more than 0.4% on the dried basis for other starches

For acetylated distarch phosphate (INS No. 1414) and hydroxypropyl distarch phosphate (INS No. 1442)

Not more than 0.14% on the dried basis for potato and wheat starch

Not more than 0.04% on the dried basis for other starches

IDENTITY

Crosslinking

Information Required

ANNEX 4: ADDITIONAL SPECIFICATIONS FOR ACETYLATED STARCHES

Version 2018

APPLIES TO

Acetylated distarch phosphate (INS No. 1414)

Starch acetate (INS No. 1420)

Acetylated distarch adipate (INS No. 1422)

Acetylated oxidized starch (INS No. 1451)

TREATMENT

This type of modified starch is obtained by esterification with acetic anhydride or vinyl acetate. Acetylation results in substitution of hydroxyl groups with acetyl esters.

- *Acetylated distarch phosphate (INS 1414)* is obtained by esterification/cross-linking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate.
- *Starch acetate (INS 1420)* is obtained by esterification of food starches with acetic anhydride or vinyl acetate
- *Acetylated distarch adipate (INS 1422)* is obtained by esterification of unmodified food starch with acetic anhydride and esterification/cross-linking with adipic anhydride
- *Acetylated oxidized starch (INS 1451)* is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride

C.A.S numbers

Acetylated distarch phosphate (INS No. 1414)
9067-33-8(Modified starch)
68130-14-3(Modified starch)
113894-91-0 (Modified amylopectin)

Starch acetate (INS No. 1420)
9045-28-7 (Modified starch)

Acetylated distarch adipate (INS No. 1422)
63798-35-6(Modified starch)
63055-36-7 (Modified amylopectin)

Acetylated oxidized starch (INS No. 1451)
68187-08-6(Modified starch)

CHARACTERISTICS

IDENTIFICATION

Specific reaction for Acetyl groups — Passes TEST

See description under TESTS

Ester groups — Passes TEST

See description under TESTS

PURITY

Acetyl groups — Not more than 2.5% on the dried basis

See description under TESTS

Vinyl acetate — Not more than 0.1 mg/kg

See description under TESTS

TEST

IDENTIFICATION TESTS

Specific reaction for acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch and converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure

Suspend about 10 g of the sample in 25 ml water. Add 20 ml of 0.4 M NaOH. After shaking for 1 h filter the starch off and evaporate the filtrate in an oven at 110°. Dissolve the residue in a few drops of water and transfer to a test tube. Add calcium hydroxide and heat the tube. If the sample is acetylated starch, acetone vapours are produced. These produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 M NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

Ester groups — The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm^{-1} which is an indication

for ester groups. The limit of detection is about 0.5% acetyl groups in the product.

PURITY TESTS

Acetyl groups

Accurately weigh about 5 g of the sample and transfer into a 250 ml conical flask. Suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 M sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 M sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30° as some starches may gelatinise). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 M hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 M hydrochloric acid required as S.

Perform a blank titration on 25.0 ml of 0.45 M sodium hydroxide, and record the volume, in ml, of 0.2 M hydrochloric acid required as B.

$$\text{Acetyl groups } \% = \frac{(\mathrm{B} - \mathrm{S}) \times \mathrm{M} \times 0.043 \times 100}{\mathrm{W}}$$

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, in grams.

ANNEX 5: ADDITIONAL SPECIFICATIONS FOR STARCHES SUBJECTED TO OXIDATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- *A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method.*

APPLIES TO

Oxidized starch (INS No. 1404)

Acetylated oxidized starch (INS No. 1451)

TREATMENT

Sodium hypochlorite is used for oxidation.

- *Oxidized starch (INS 1404)* is obtained by treatment of food starch with sodium hypochlorite.
- *Acetylated oxidized starch (INS 1451)* is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride.

Oxidation involves the deliberate production of carboxyl groups.

C.A.S number

Oxidised starch (INS No. 1404)
65996-62-5 (modified starch)
113894-86-3 (modified amylopectin)

Acetylated oxidised starch (INS No. 1451)
68187-08-6

CHARACTERISTICS

IDENTIFICATION

Test for hypochlorite oxidized starch

Passes test

See description under TESTS

PURITY

Carboxyl groups (Vol. 4)

Not more than 1.3% on the dried basis

Residual hypochlorite

Information required

TESTS

IDENTIFICATION TESTS

Test for hypochlorite oxidized starch

Principle

Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue. The test is not suitable for slightly oxidized potato starch due to the presence of phosphate groups.

Procedure

50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

ANNEX 6: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED WITH OCTENYLSUCCINIC ANHYDRIDE

Version 2018

APPLIES TO Starch sodium octenylsuccinate (INS No. 1450)

TREATMENT Octenylsuccinic anhydride can be used for the esterification and either sodium hydroxide or sodium carbonate as a pH buffer for neutralisation.

C.A.S numbers Starch sodium octenylsuccinate
66829-29-6(Modified starch)
52906-93-1(Modified starch)
125109-81-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

Octenylsuccinyl groups: Not more than 3% on the dried basis

See description under TESTS

Residual free octenylsuccinic acid: *Not more than 0.3% on the dried basis*

See description under TESTS

PURITY TEST

Octenylsuccinate groups and residual free octenylsuccinic acid in Starch sodium octenyl succinate

Principle
Residual free octenylsuccinic acid in the sample is extracted and determined by HPLC/UV. Total octenylsuccinic content is determined using the same method after hydrolysis of the sample. Octenylsuccinate ester groups on the modified starch are calculated by subtraction of the residual free octenylsuccinic acid from the total.

Standard and Reagents

Octenylsuccinic anhydride:
2-Octen-1-ylsuccinic anhydride, mixture of *cis* and *trans* (>97%) (CAS: 42482-06-4)

0.1 N potassium hydroxide:
Weigh1.4 g of potassium hydroxide, dissolve in water and dilute to 250 ml

0.073 mol/l phosphoric acid:

Dilute 1 ml of phosphoric acid (85%, density 1.686g/cm^3) to 200 ml with water.

Preparation of standard solutions

Accurately weigh about 20 mg of octenylsuccinic anhydride, add 10 ml of 0.1N potassium hydroxide, stopper and heat at 80° for 3 hours. After cooling, add 8 ml of 0.073mol/l phosphoric acid and dilute with water to 20 ml. Pipette 2 ml of this solution into a 20 ml volumetric flask and dilute with water. Pipette 1 ml, 2 ml, 5 ml, and 10 ml of the resulting solution into four separate 20-ml volumetric flasks, and dilute each to volume with water to prepare standards of 5 µg/ml, 10 µg/ml, 25 µg/ml and 50 µg/ml respectively.

Preparation of test solution A (for residual octenylsuccinic acid):

Accurately weigh about 0.1 g of sample, add 20 ml of methanol, and shake for 18 hours or more. Centrifuge the mixture at about 3000 rpm for 5 minutes, pipette 10 ml of the supernatant, and evaporate to dryness under vacuum at 40°. Dissolve the residue and dilute with water in a 5 ml volumetric flask.

Preparation of test solution B (for total octenylsuccinic acid):

Accurately weigh about 20 mg of sample, dissolve in 10 mL of 0.1N potassium hydroxide, stopper and heat at 80°for 3 hours. After cooling, add 8 ml of 0.05mol/l phosphoric acid, dilute with water to 20 ml.

Procedure

HPLC operating conditions

- Column: A octadecylsilanized silica gel column (250 mm x 4.6 mm, 5µm) (L-Column ODS-V CERI or equivalent)
- Column temperature: 40°
- Detector: UV at 205 nm
- Mobile phase: A 1:1 mixture of 0.1% (v/v) phosphoric acid solution / acetonitrile
- Injection volume: 20µl
- Flow rate: Adjust the retention time of the main peak to about 9 minutes.

Inject the test solution A and B and the standard solutions into an HPLC under the same conditions.

Measure the sum of the peak areas of two main peaks of *cis*- and *trans*-2-octenylsuccinic acid for each standard solution, and prepare a standard curve for octenylsuccinic anhydride from the sums obtained and the concentrations of octenylsuccinic anhydride in the standard solutions. Measure the sum of the peak areas of two main peaks for the test solutions A and B. Determine the concentration of

octenylsuccinic acid (μg/ml) in the test solutions A and B from the standard curve, and calculate residual and total octenylsuccinic acid, respectively. The value of octenylsuccinate groups in the sample is calculated by the following formula:

Calculation:

$$Residual\ free\ octenylsuccinic\ acid\ \% = C_{OS}\ x\ 1.086\ /\ W_r\ x\ 100$$

$$Total\ octenylsuccinic\ acid\ \% = C_{os}\ x\ 1.086\ /\ W_s\ x\ 500$$

Where

- 1.086 is the molecular weight of octenylsuccinic acid divided by the molecular weight of octenylsuccinic anhydride
- C_{os} is the octenylsuccinic anhydride concentration (μg/ml);
- W_r or W_s is the dry-basis weight of the sample (g).

Content (%) of octenylsuccinyl groups =
Content of total octenyl succinic acid – Content of residual octenylsuccinic acid.

ANNEX 7: ADDITIONAL SPECIFICATIONS FOR STARCHES ETHERIFIED WITH PROPYLENE OXIDE

(Version 2018 - TENTATIVE)

Information is required on:

- *A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of Hydroxypropyl starch using the method.*

APPLIES TO

Hydroxypropyl starch (INS No. 1440)

Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

Propylene oxide is used for etherification.

- *Hydroxypropyl starch (INS No. 1440)* is obtained by etherification of unmodified food starch with propylene oxide.
- *Hydroxypropyl distarch phosphate (INS No. 1442)* is obtained by esterification of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide.

Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether.

C.A.S numbers

Hydroxypropyl starch(INS No. 1440)
9049-76-7(Modified starch)
74315-67-6 (Modified amylopectin)

Hydroxypropyl distarch phosphate (INS No. 1442)
53124-00-8(Modified starch)
113894-92-1 (Modified amylopectin)

CHARACTERISTICS

IDENTIFICATION

Hydroxypropyl ether groups

Passes test

See description under TESTS

PURITY

Hydroxypropyl groups

Not more than 7.0% on the dried basis

See description under TESTS

Propylene chlorohydrins

Not more than 1 mg/kg

See description under TESTS

TESTS

IDENTIFICATION TESTS

Hydroxypropyl ether groups[2]

Ninhydrin reagent

A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure

Weigh 100 mg of the sample into a 100-ml volumetric flask and add 12.5 ml of 2 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake).

A violet colour develops only in the modified sample within 5 min due to the presence of hydroxypropyl groups (starch ether).For all other non-hydroxypropyl treated starches a light pink colour is observed.

PURITY TEST

Hydroxypropyl groups

Ninhydrin reagent

A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure

Accurately weigh 50 - 100 mg of the sample into a 100-ml volumetric flask and add 25 ml of 1 N sulfuric acid. Prepare a sample of unmodified starch of the

[2] USP29-NF34: U.S. Pharmacopeial Convention, Hydroxylpropyl corn starch monograph, 2015. Reproduced from the USP-NF with permission from The U.S. Pharmacopeial Convention (USP)

same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. If necessary, dilute the sample further to assure the presence of no more than 4 mg of hydroxypropyl group per 100 ml, and then dilute the blank starch in the same proportion. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). Immediately transfer portions of the solutions to 1-cm cells and after exactly 5 min, measure the absorption (A) at 590 nm, using the starch blank as the reference. Prepare a calibration curve with 1-ml aliquots of standard aqueous solutions, containing 10, 20, 30, 40 and 50 µg of propylene glycol per ml.

Calculation

$$Hydroxypropyl\ groups\ (\%) = \frac{C \times 0.7763 \times 10 \times F}{W}$$

where

- C is the amount of propylene glycol in the sample solution read from the calibration curve (µg/ml);
- F is the dilution factor (if a further dilution has been necessary); and
- W is the weight of sample (mg).

Propylene chlorohydrins

Principle

Propylene chlorohydrins (1-chloro-2-propanol and 2-chloro-1-propanol) in sample are determined by capillary gas chromatography.

Standards and Reagents

propylene chlorohydrins

Preparation of standard addition calibration curve

Accurately weigh about 50 mg propylene chlorohydrins, and dilute with water to 100 ml. Dilute 10 ml of this solution to 100 ml with water to make a standard stock solution (50 µg/ml). Take four Erlenmeyer flasks and weigh 50.0 g of sample in each one. Add 125 ml of 1 M sulfuric acid in each one. Add 0.5 ml, 1 ml, 2 ml, or 5 ml of standard stock solution, to the 1st, 2nd, etc flasks respectively. Proceed as directed for the test solution, beginning

with “and swirl the flask to disperse the contents,” to prepare the standard solutions of added concentration 5, 10, 20 and 50 µg/ml respectively.

Preparation of test solution

Accurately weigh 50 g of sample into an Erlenmeyer flask, add 125 ml of 1 M sulfuric acid, and swirl the flask to disperse the contents. Stopper loosely, heat in a water bath at 100°for 10 min, mix the contents well, and heat for an additional 30 min. For starches that are not easy to hydrolyze, such as wheat starch, heating time should be longer (90 min). Cool to room temperature, adjust the pH to 7 with 25% sodium hydroxide solution, and filter with suction through a glass-fiber filter paper. Wash the flask and the residue on the filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, stir for 5–10 min to dissolve, and transfer the solution into a separating funnel. Wash the flask with 25ml of water, and add the washings to the funnel. If precipitate remains, stir well with a small amount of water to dissolve it completely, and add the solution to the funnel. Extract five times with five 50ml portions of diethyl ether. Combine the diethyl ether extracts, add 3 g of anhydrous sodium sulfate, let it stand for a few minutes and filter through a filter paper. Wash the flask and the filter paper with 25 ml of diethyl ether, and combine the washings with the filtrate. Evaporate to 4 ml in a water bath at about 40° under atmospheric pressure, cool, transfer to a 5 ml volumetric flask and add diethyl ether to the exact volume.

Procedure

GC operating conditions

- GC equipped with a flame ionization detector (FID).
- Column: A fused silica column coated with polyethylene glycol (30 m x 0.25 mm i.d., 0.25 µm) (Inert Cap WAXGL Sciencesor equivalent）
- Carrier gas: N_2 or He
- Flow rate: Adjust the retention time of 1-chloro-2-propanol to about 15 min
- Column temperature: 40°-for 2 min; heat at 5°/min to 80°, keep for 8 min, heat at 25°/min to 230°, keep for 5 min
- Injector temperature: 150°
- Detector temperature: 230°
- Split-less（purge start: 1 min after injection）

Analyse 1-μl portions of the test solution and the standard solutions by gas chromatography, using the operating conditions given above. Prepare a standard addition curve: Plot in the y axis the sum of the peak areas corresponding to 1-chloro-2-propanol and 2-chloro-1-propanol in the chromatographs and in the x-axis the added concentration of propylene chlorohydrins in the standard solution. For the test solution the added concentration is equal to 0. A linear calibration curve should be obtained. Extrapolate to 0 in the y axis. The concentration (μg/ml) of propylene chlorohydrins in the test solution is equal to the absolute value of the concentration at the point where the curve intercepts the x axis (C_t). Determine the content of propylene chlorohydrins in the sample using the following formula:

Calculation

$$\text{Content (mg/kg) of Propylene chlorohydrins} = C_t \times 5 / W$$

where

- C_t: amount of propylene chlorohydrins in test solution (μg/mL);
- W: mass of sample (g, on the dried weight basis)

ANNEX 8: ADDITIONAL SPECIFICATIONS FOR STARCHES CROSSLINKED WITH ADIPIC ANHYDRIDE

(Version 2018 – Tentative)

Information is required on:

- *A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches*
- *Levels of free adipic acid in at least 5 representative batches*

APPLIES TO Acetylated distarch adipate (INS No. 1422)

TREATMENT Adipic anhydride can used for esterification and crosslinking. In cases of cross-linking, where adipic anhydride connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = CO-$(CH_2)_4$-CO and starch refers to the linear and/or branched structure.

C.A.S numbers Acetylated distarch adipate
63798-35-6(Modified starch)
63055-36-7 (modified amylopectin)

CHARACTERISTICS

PURITY

Adipate groups Not more than 0.135% on the dried basis

See description under TESTS

Free adipic acid *Information Required*

See description under TESTS

PURITY TEST

Adipate groups and free adipic acid Determine by gas chromatography after derivatization

Principle
Free adipic acid in the sample is extracted and determined by capillary gas chromatography after trimethylsilyl-derivatization. Total adipic acid is determined using the same method after hydrolysis of the sample and adipate groups are calculated by subtraction of the free adipic acid from the total.

Standards and Reagents
Adipic acid (>99%)
Glutaric acid (>99%)
Starch, unmodified (of the same botanical origin as the sample)
Sodium hydroxide solution (4N): weigh 40g of NaOH, dissolve in water and dilute to 250 ml.
Concentrated HCl (36%)
Ethyl acetate
Sodium sulphate, anhydrous
N,O-Bis(trimethylsilyl)trifluoroacetamide
Pyridine

Internal standard solution (1 mg/ml)
Accurately weigh 0.1 g of glutaric acid, dissolve in water and dilute to 100 ml.

Standard stock solution (1 mg/ml)
Accurately weigh 0.1 g of adipic acid, dissolve in 90 ml of warm water, cool to room temperature, dilute to 100 ml and mix.

Working standard solutions (0.02, 0.1, 0.2 and 0.4 mg/ml)
Pipette 1, 5, 10, and 20 ml of the standard stock solution in four separate 50 ml volumetric flasks, and dilute with water.

Procedure
Preparation standard curve solutions
Weigh 1.0 g of starch into each of four Erlenmeyer flasks, add 50 ml of water and 1 ml of internal standard solution. Add 5 ml each of the four working standard solutions, respectively. Stopper the flask and shake them well to disperse the starch, add 50 ml of 4N sodium hydroxide solution, and shake for 5 min. Place the flasks in a water bath, at room temperature, and add cautiously 20 ml of conc. hydrochloric acid. Cool, and quantitatively separately transfer the contents of the flasks into four separation funnels with a little amount of water. Extract three times with 100 ml of ethyl acetate each time. Collect the ethyl acetate layers separately in four dry Erlenmeyer flasks, add 20 g of anhydrous sodium sulphate, allow to stand for 10 min with occasional shaking, and filter into a rotary evaporator flask. Wash the Erlenmeyer flask and the residue on the filter paper twice with a small quantity of ethyl acetate, and combine the washings with the filtrate. Evaporate the ethyl acetate under a reduced pressure of 6.7 kPa at a temperature below 40°. Remove the remaining ethyl acetate completely by nitrogen stream. The evaporation of ethyl acetate should be effected as quickly as possible. Successively add 2 ml of pyridine and 1 ml of N,O-bis(trimethylsilyl)trifluoroacetamide to the residue and stopper the flask. Allow the solution to stand for 1 hour, transfer 2 ml of it into a GC vial, and immediately stopper tightly. Use these solutions to construct standard curve (Internal standard 1 mg/g starch, standards 0.1, 0.5, 1 and 2 mg/g starch respectively)

Preparation of test solution A (for residual free adipic acid)
Weigh accurately about 5 g of sample into an Erlenmeyer flask, add 100 ml of water and 1 ml of the internal standard solution. Shake well for 1 hour, and filter through a 0.45 μm membrane filter. To the filtrate, add exactly 1 ml of hydrochloric acid (in the case of pre-gelatinized starch or water-soluble starch, directly add 1 ml of hydrochloric acid to the resulting suspension without filtering), and transfer into a separation funnel. Proceed as directed for the preparation of standard solutions, beginning with "…and wash the inside of the flask with a little amount of water into the funnel." Use this solution for the determination of residual free adipic acid (Internal standard 1 mg/ 5 g starch).

Preparation of test solution B (for total adipic acid)
Weigh accurately about 1 g of sample into an Erlenmeyer flask, add 50 ml of water and exactly 1 ml of the internal standard solution. Shake the mixture well to disperse the starch, add 50 ml of 4N sodium hydroxide solution and shake well for 5 minutes. Place the flask in a water bath at room temperature, and add cautiously 20 ml of concentrated hydrochloric acid. After cooling, transfer the contents in the flask into a separation funnel. Proceed as directed for the preparation of standard solution, beginning with "…and wash the inside of the flask with a little amount of water into the funnel." Use this solution for the determination of total adipic acid (Internal standard 1 mg/g starch).

Procedure
GC operating conditions

- GC equipped with a flame ionization detector (FID) Column:A fused silica column coated with a mixture of 50% diphenyl and 50% dimethylpolysiloxane (15 m x 0.25 mm i.d., 0.25 μm)
- Carrier gas: He
- Column flow 1.0 ml/min.
- Column temperature: 120°-5 min-5°/min-150° (Glutaric and adipic acids elute at about 5 min and 8 min respectively)
- Injector temperature: 250°
- Detector temperature: 250°
- Injection volume 1μl
- Split ratio: 30:1

Inject standard curve solutions into the capillary GC under the conditions indicated and construct a standard curve using the peak area ratios of adipic acid and glutaric acid against the amounts of adipic acid in the standard solutions (in g). Inject the test solution A and Band obtain the peak area ratio of adipic acid to glutaric acid for each of the test solutions A and B.

Determine the amount of adipic acid in each test solution from the standard curve and calculate the percent of adipate groups using the following formula:

Free adipic acid, %w/w = [CF/MF] x 100

Adipate groups. %w/w = [CT/MT–CF/MF] x 100

where

CT = amount of the total adipic acid in the test solution B (g)

CF = amount of the free adipic acid in the test solution A (g)

MT =mass of sample in the test solution for the determination of total adipic acid (g, on the dried weight basis)

MF =mass of the sample in the test solution for the determination of free adipic acid (g, on the dried weight basis)

NEUTRAL METHACRYLATE COPOLYMER (TENTATIVE)

New tentative specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). An ADI of "not specified" was established at 86th JECFA (2018).

Information required on:

- *A validated method for the assay of neutral methacrylate copolymer (e.g., quantitative IR)*
- *Performance characteristics (method validation data) of the assay method*
- *Assay and monomers data on at least five batches of products currently available in commerce*

SYNONYMS

E 1206, INS No. 1206, Ethyl acrylate methyl methacrylate polymer, Ethyl acrylate methyl methacrylate polymer; Ethyl acrylate polymer with methyl methacrylate, Methyl methacrylate ethyl acrylate polymer, Methyl methacrylate polymer with ethyl acrylate.

DEFINITION

Neutral Methacrylate Copolymer is a copolymer comprised of the monomers ethyl acrylate and methyl methacrylate in the molar ratio of 2:1. The copolymer is manufactured by emulsion polymerization of the monomers with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low-molecular weight substances and coagulum. The copolymer is standardized as a 30% aqueous dispersion with polyethylene glycol monostearyl ether. The copolymer dispersion may contain the residual monomers (methyl methacrylate and ethyl acrylate). Neutral methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes

Chemical name

Poly(ethyl acrylate-co-methylmethacrylate)

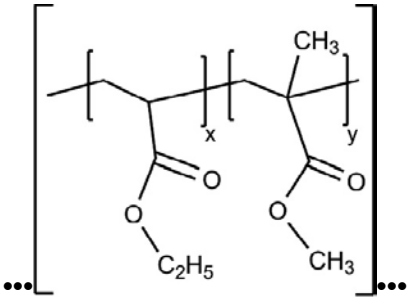
C.A.S. number

9010-88-2

Chemical formula

Poly[(CH_2:$CHCO_2CH_2CH_3$)-co-(CH_2:$C(CH_3)CO_2CH_3$)]

Structural formula



The above formula is provided for illustrative purposes; in this copolymer no definitive structural unit can be defined.

Formula weight 600,000 (weight-average), 220,000 (number-average)

Assay Information required

DESCRIPTION Commercial form (30% aqueous dispersion) is a low viscosity milky-white liquid

FUNCTIONAL USES Coating agent, binding agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Viscosity (Vol. 4) Not more than 50 mPa.s

Determine viscosity using Brookfield viscometer at 20° and 300 pm using UL adapter.

pH (Vol 4) 5.5 – 8.6

Infrared absorption The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.

Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol 4) 68.5 – 71.5% (110°, 3 h)

Sulfated ash (Vol. 4) Not more than 0.4%

Test 5 g of the sample (Method I)

Residual solvents (Vol. 4) — Methanol: Not more than 100 mg/kg

Residual monomers — Ethanol: Not more than 1,000 mg/kg

Methyl methacrylate: Not more than 50 mg/kg

Ethyl acrylate: Not more than 20 mg/kg

See description under TESTS

Lead (Vol. 4) — Not more than 1.0 mg/kg

Determine using a method 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").

Microbiological criteria (Vol. 4) — Total plate count: Not more than 1,000 cfu/g

Yeast and moulds: Not more than 100 cfu/g

Coliforms: Negative in 10 g

TESTS

PURITY TESTS

Residual monomers — Determined by liquid chromatography (Vol. 4)

Standards and Reagents:

- Acetonitrile: UV absorption: A_{max} of 1% at 190 nm
- Tetrahydrofuran and deionized water:
- Sodium perchlorate (35g/l)
- Standards: Ethyl acrylate and methyl methacrylate (>99%)

Preparation of mixed standard solutions
Stock mixed standard solution (200 µg/ml):
Accurately weigh about 10 mg each of ethyl acrylate and methyl methacrylate, dissolve in tetrahydrofuran and make up to 50 ml with tetrahydrofuran in a volumetric flask.

Intermediate mixed standard solution-1 (20 µg/ml):
Dilute 1.0 ml of stock mixed standard solution to 10 ml with tetrahydrofuran in a volumetric flask.

Intermediate mixed standard solution-2 (2 µg/ml):
Dilute 1.0 ml of intermediate mixed standard solution-1 to 10 ml with tetrahydrofuan in a volumetric flask.

Working mixed standard solution (0.67 µg/ml):
To 10 ml of Intermediate mixed standard solution-2 add 5 ml of sodium perchlorate and mix. Dilute 5 ml of this mixture to 10 ml with deionized water.

Preparation Sample Solution
Accurately weigh approximately1.0 g of sample, dissolve in tetrahydrofuran and dilute to 50.0 ml in a volumetric flask. To 5 ml of sodium perchlorate solution, add 10 ml of sample solution drop wise, whilst stirring continuously. Centrifuge and filter the clear supernatant. Dilute 5 ml of this mixture to 10 ml with deionized water.

Procedure
Use a HPLC with diode array/UV detector at 205 nm
Column: Octadecylsilyl silica gel (12 cm x 4.6 mm i.d., 5-10 µm.)
Injection volume: 50 µl
Mobile phase: Acetonitrile:Water (15:85)
Flow rate: 2 ml/min

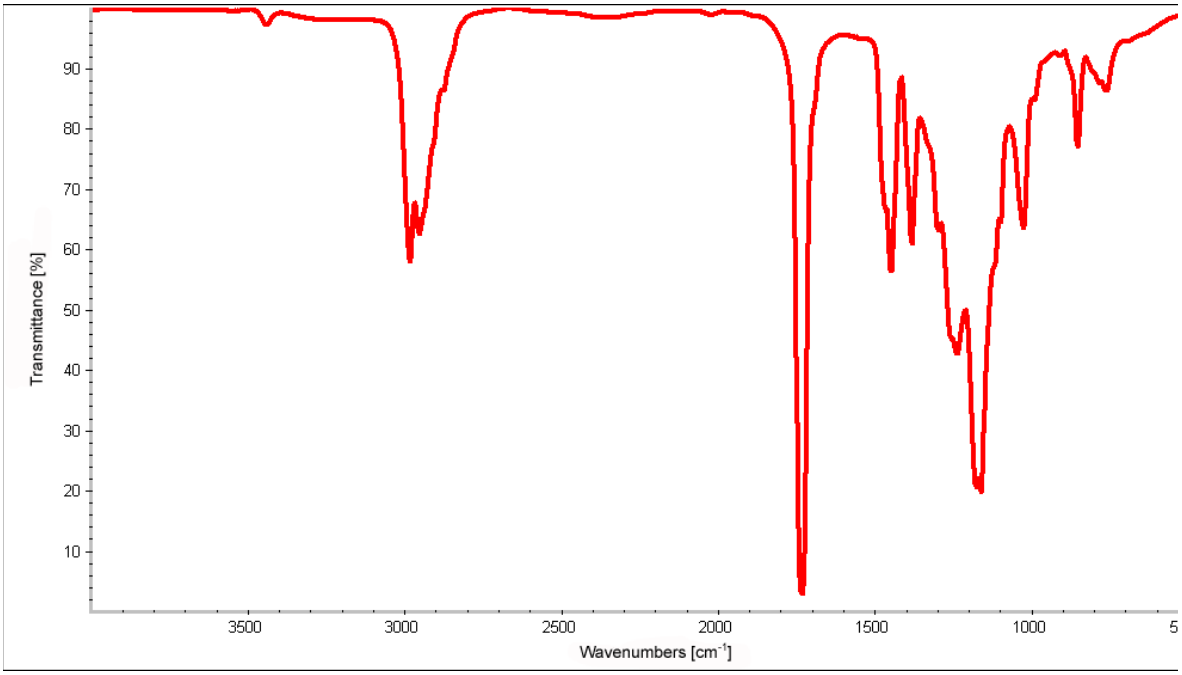
Inject separately 50 µl each of working mixed standard solution and sample solution. Calculate the amount of each monomer in the sample from the peak areas obtained in the chromatograms of working mixed standard solution (rR) and sample solution (rS); amount of standard in the injected solution (R, µg) and weight of sample in injected sample solution (W, g)

$$\text{Amount of each monomer } (\mu g/g) = \frac{rS \times R}{rR \times W}$$

Total monomers in the sample (µg/g) = Sum of monomers in the sample

METHOD OF ASSAY Information Required

Appendix: Infrared spectrum of neutral methacrylate copolymer



SPIRULINA EXTRACT (TENTATIVE)

New specifications prepared at the 86th JECFA (2018), published in FAO JECFA Monograph 22 (2018). A temporary ADI "not specified" was established at the 86th JECFA (2018).

Information Required on:

- *Full compositional characterization of commercial products in both liquid and powder forms.*
- *Full compositional characterization of the aqueous extract before formulation/standardization.*
- *Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data).*
- *Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).*

SYNONYMS INS 134; Spirulina colour

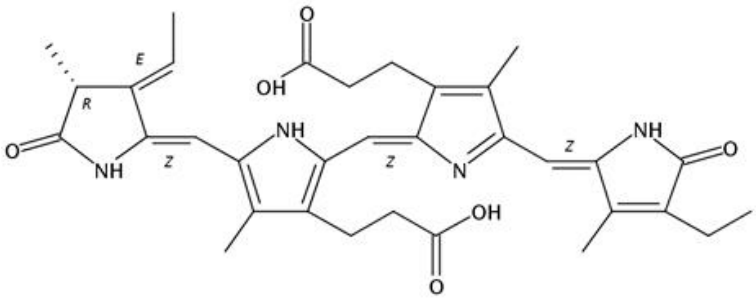
DEFINITION Spirulina extract is obtained by aqueous extraction of the biomass of *Arthrospira platensis*, an edible cyanobacterium. The organism is cultivated and harvested under conditions that prevent the growth of other cyanobacteria and the production of microcystins. The material extracted from the biomass is further treated by steps that may include pH adjustment, centrifugation, filtration, concentration, sterilization, drying, and dilution to the desired degree of pigment concentration. The main colouring principles are two phycobiliproteins, C-phycocyanin and allophycocyanin, which are water-soluble pigment-protein complexes where the chromophore is covalently bonded to the protein. Extracts may also contain trace amounts of chlorophyll, beta-carotene, and other carotenoids. Spirulina extract may contain peptides, other proteins, carbohydrates and minerals. Commercial products are formulated in liquid and powder forms.

C.A.S. number 20298-86-6

(Phycocyanobilin; 3-[(2Z,5E)-2-[[3-(2-carboxyethyl)-5-[(Z)-[(3E,4R)-3-ethylidene-4-methyl-5-oxopyrrolidin-2-ylidene]methyl]-4-methyl-1H-pyrrol-2-yl]methylidene]-5-[(4-ethyl-3-methyl-5-oxopyrrol-2-yl)methylidene]-4-methylpyrrol-3-yl]propanoic acid)

Chemical formula $C_{33}H_{38}N_4O_6$ (Phycocyanobilin)

Structural formula



Phycocyanobilin

Formula weight	586.68 (Phycocyanobilin)
Assay	Total phycocyanins as the sum of C-phycocyanin and allophycocyanin not less than declared. See description under TESTS
DESCRIPTION	Clear blue liquid or blue powder
FUNCTIONAL USES	Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water. Insoluble in ethanol.
Colour Value	Not less than declared (15 to 300 for powdered products on the dried basis and 10 to 70 for liquid products). See description under TESTS

PURITY

Loss on drying (Vol. 4)	Not more than 6% for the powdered product (105°, 4h)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

Cadmium (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Mercury (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	Total (aerobic) plate count: less than 1000 CFU/g Yeast and moulds: less than 100 CFU/g Coliforms: absent in 10 g *Salmonella spp*.: absent in 25 g *S. aureus*: absent in 10 g
Microcystins	Less than 0.5 µg/g as microcystin-LR (dried basis) See description under TESTS

TESTS

PURITY TESTS

Microcystins	Principle Determine microcystins by enzyme linked immunoassay (ELISA) under the following conditions: Reagent Methanol/water (75:25, v/v)

Equipment
Use a commercially available ELISA kit with cross reactivity for microcystin-LR and other microcystins.

Sample preparation
In the absence of other instructions provided by the ELISA kit manufacturer, follow the procedure presented here.

Dry an appropriate amount of spirulina extract. Homogenize 3.0 g of the dried material in 20.0 ml of the methanol/water reagent for 20 minutes. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Transfer the supernatant into a glass flask. Add 10.0 ml of the methanol/water reagent to the homogenizer and homogenize the residue for 30 seconds. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Combine the supernatants and dilute with water to a concentration within the range indicated by the ELISA kit manufacturer.

Procedure
Follow the instructions provided by the ELISA kit manufacturer.

Colour Value

For the purpose of this specification, Colour Value is based on the absorbance of a buffered solution at 618 nm.

Reagent
Sodium phosphate buffer (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure
Transfer 330 mg of spirulina extract into a 100 ml volumetric flask and dilute to volume with water. Transfer 10 ml of the solution into a second 100 ml volumetric flask and dilute to volume with the sodium phosphate buffer (100 mM, pH 6.0). Determine the absorbance (A_{618}) of the solution in a 1-cm cell at 618 nm with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference.

Calculate the Colour Value of the spirulina extract as follows:

$$\text{Colour Value} = A_{618} \times 100 / W_1$$

Where
W_1 is the weight of spirulina extract taken, in g

METHOD OF ASSAY Principle

Determine total phycocyanins as the sum of C-phycocyanin and allophycocyanin under the following conditions:

Reagent
Sodium phosphate buffer (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure
Transfer 100 mg of spirulina extract into a 25 ml volumetric flask and dilute to volume with sodium phosphate buffer (100 mM, pH 6.0). Sonicate the mixture for 30 minutes maintaining the temperature at 8°. Incubate at 30° for 8 h, shaking manually every hour. Mix the contents of the flask and transfer to a centrifugation tube; centrifuge at 3500 rpm for 4 minutes. Determine the absorbance of the supernatant in a 1-cm cell at 620 nm (A_{620}) and 650 nm (A_{650}) with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference. The dilution should be adjusted with additional buffer, if needed, to obtain absorbance values of 0.2 to 0.6 at 620 nm.

Calculate the C-phycocyanin content of the spirulina extract (%, w/w) as follows:

$$T_{cPC} = (0.162 \times A_{620}) - (0.098 \times A_{650}) \times V_1 \times 100 / W_1$$

Where
W_1 is the weight of spirulina extract taken, in mg
V_1 is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the allophycocyanin content of the spirulina extract (%, w/w) as follows:

$$T_{aPC} = (0.180 \times A_{620}) - (0.042 \times A_{650}) \times V_1 \times 100 / W_1$$

Where
W_1 is the weight of spirulina extract taken, in mg
V_1 is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the total phycocyanin content of the spirulina extract as follows:

$$T_{PC} = T_{cPC} + T_{aPC}$$

SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS

At the 86th meeting, the Committee prepared specifications of identity and purity of 69 flavourings in 8 sub-categories for the following numbers: 380.1, 380.2, 427, 433, 619, 973-975, 980-982, 1480, 1491-1526, 2103-2105, 2123, 2235-2244, 2246-2255.

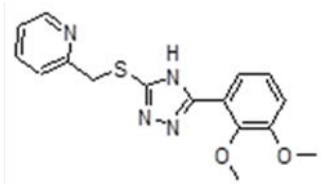
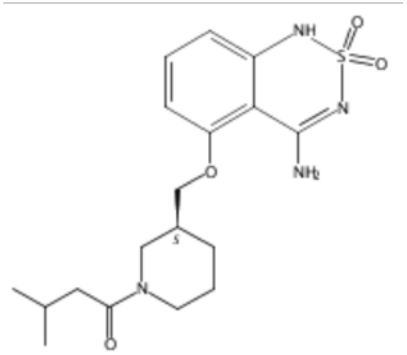
Information on specifications for flavouring agents is given in the tables, most of which are self-explanatory: Name; Chemical name (Systematic name, normally IUPAC name); Synonyms; Flavour and Extract Manufacturers' Association of the United States (FEMA) No; FLAVIS (FL) No; Council of Europe (COE) No; Chemical Abstract Service Registry (CAS) No; Chemical formula (Formula); Molecular weight (MW); Physical form/Odour; Solubility; Solubility in ethanol, Boiling point (B.P. ºC – for information only); Identification test (ID) referring to type of test (NMR: Nuclear Magnetic Resonance spectrometry; IR: Infrared spectrometry; MS: Mass spectrometry); Assay min % (Gas chromatographic (GC) assay of flavouring agents); Acid value max; Refractive index (R.I.) (at 20º, if not otherwise stated); Specific gravity (S.G) (at 25º, if not otherwise stated). The field called "Other requirements" contains four types of entry:

1. Items that are additional requirements, such as further purity criteria or other tests.
2. Items provided for information, for example the typical isomer composition of the flavouring agent. These are not considered to be requirements.
3. Substances which are listed as Secondary Constituents (SC) which have been taken into account in the safety evaluation of the named flavouring agent. If the commercial product contains less than 95% of the named compound, it is a requirement that the major part of the product (i.e. not less than 95% is accounted for by the sum of the named compound and one or more of the secondary constituents.
4. Information on the status of the safety evaluation.

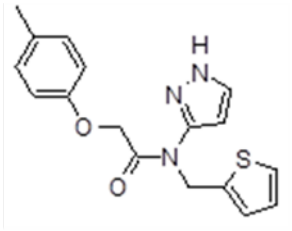
The fields named Session/Status contain the number of the meeting at which the specifications were prepared and the status of the specification. All specifications prepared at the 86th meeting were assigned full status.

The flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents and a list for conclusions in alphabetical order is given in Annex I.

GROUP 1: MISCELLANEOUS NITROGEN-CONTAINING SUBSTANCES

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / CE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2235	**2-(((3-(2,3-Dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)thio)methyl)pyridine**	4798	$C_{16}H_{16}N_4O_2S$	Phosphate buffer, pH 7.1 = 0.30 mM	MS, 1H-NMR, 13C-NMR, IR	NA	mp: 114.0 - 116.0 º
Draft	Pyridine, 2-(((3-(2,3-dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)thio)methyl)		328.39	Soluble	>98 %	NA	
	2-((5-(2,3-dimethoxyphenyl)-2H-1,2,4-triazol-3-ylthio)methyl)pyridine		White to off-white solid				
							
86		902136-79-2					
2236	**(S)-1-(3-(((4-Amino-2,2-dioxido-1H-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one**	4802	$C_{18}H_{26}N_4O_4S$	Soluble	MS, 1H-NMR, 13C-NMR, IR	NA	mp: 236-238 º; <5% R-enantiomer
Draft	(S)-1-(3-(((4-amino-2,2-dioxido-1H-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one		394.49	Soluble	>95 %	NA	
			Off-white solid				
							
86		1469426-64-9					

2237	**2-(4-Methylphenoxy)-N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)acetamide**	4809	$C_{17}H_{17}N_3O_2S$	Slightly soluble at pH 2.8	MS, 1H-NMR, 13C-NMR, IR	NA	mp: 115-116.5 °C
Draft	N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)-2-(4-tolyloxy)acetamide	16.133	327.40	Soluble	99%	NA	
			White to off-white solid				

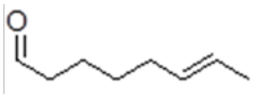
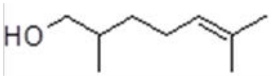


86 1374760-95-8

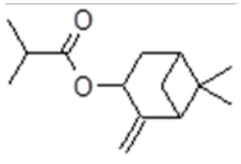
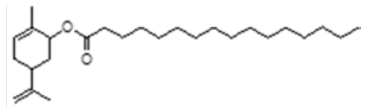
GROUP 2: SATURATED ALIPHATIC ACYCLIC BRANCHED-CHAIN PRIMARY ALCOHOLS, ALDEHYDES, AND ACIDS

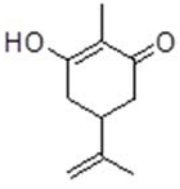
JECFA No.	Name	FEMA	Chemical Formula	Solubility Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W		Assay min %	S.G. (25°)	
	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		Information required
Session		CAS					
2238	**8-Methyldecanal**	4795	$C_{11}H_{22}O$	very slightly soluble	MS, 1H-NMR, 13C-NMR	1.4224-1.4421	
Draft	8-methyldecanal		170.29	Soluble	95%	0.879-0.919 (20 °)	
			colorless liquid/ citrus/green odor	222-223 º	10		
86		127793-88-8					
2239	**8-Methylnonanal**	4803	$C_{10}H_{20}O$	very slightly soluble	MS, 1H-NMR, 13C-NMR	1.4256 - 1.4260	
Draft	8-Methylnonanal		156.27	Soluble	>95%	0.8227 - 0.8231 (20 °)	
	Isodecanal, Isodecaldehyde		Colorless, transparent liquid/Sweet aroma of fruit with green notes	196-197 º			
86		3085-26-5					

GROUP 3: LINEAR AND BRANCHED-CHAIN ALIPHATIC, UNSATURATED, UNCONJUGATED ALCOHOLS, ALDEHYDES, ACIDS, AND RELATED ESTERS

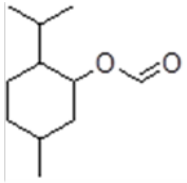
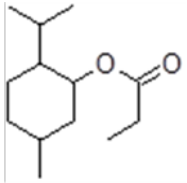
JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / CE CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2240 Draft	**trans-6-Octenal** (E)-Oct-6-enal (E)-6-Octenal; (6E)-Octenal	4787	$C_8H_{14}O$ 126.20 Colorless oil / Green, melon-like aroma	Slightly soluble Soluble 171-172º	1H-NMR, 13C-NMR 95%	1.4377 0.8536 (20 º)	
							
86		63196-63-4					Secondary component: 1-6% 2,6-dimethyl-5-heptenal (No. 349) The sum of 2,6-dimethyl-5-heptenol and 2,6-dimethyl-5-heptenal is ≥ 95%
2241 Draft	**2,6-Dimethyl-5-heptenol** 2,6-Dimethylhept-5-en-1-ol 2,6-dimethyl-5-hepten-1-ol, Melonol	4789	$C_9H_{18}O$ 142.24 Colorless to light yellow, transparent liquid/Floral fruity aroma reminiscent of melon	Slightly soluble Soluble 204-206 °	MS, 1H-NMR, 13C-NMR >90%	1.428-1.459 0.794-0.904	
							
86		4234-93-9					

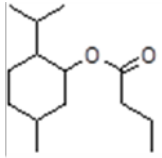
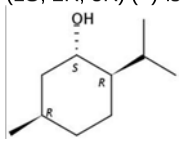
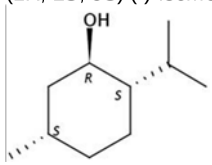
GROUP 4: CARVONE AND STRUCTURALLY RELATED SUBSTANCES

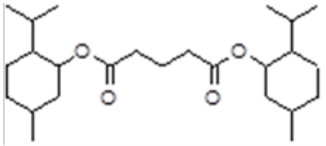
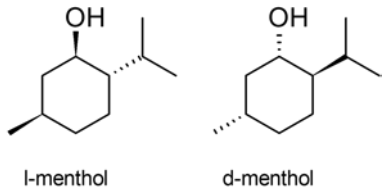
JECFA No. / Status / Session	Name / Chemical Name / Synonyms / CAS	FEMA / FLAVIS / CE	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2242	**Pinocarvyl isobutyrate**	4525	$C_{14}H_{22}O_2$	Practically insoluble to insoluble	MS	1.445-1.450	
Draft	6,6-dimethyl-2-methylidenebicyclo[3.1.1]heptan-3-yl 2-methylpropanoate Propanoic acid, 2-methyl-, 6,6-dimethyl-2-methylenebicyclo[3.1.1]hept-3-yl ester		222.32 Viscous colourless to slight/pale yellow liquid / Warm woody balsamic aroma with fruity notes	Soluble 264-266 º	>95%	0.912-0.915	
							
86		929116-08-5					mp: 144-145 º; Mixture of (2R,4S)-carvyl palmitate and (2S,4S)-carvyl palmitate
2243	**Carvyl palmitate**	4515	$C_{26}H_{46}O_2$	Practically insoluble to insoluble	MS, 1H-NMR	NA NA	
Draft	5-Isopropenyl-2-methyl-2-cyclohexen-1-yl palmitate Carvyl hexadecanoate		390.64 Waxy solid/Rich fatty spearmint to caraway aroma	Soluble	>95%		
							
86		929222-96-8					

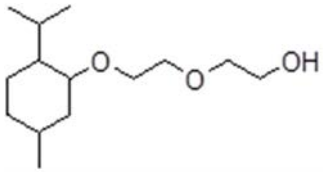
2244	**6-Hydroxycarvone**	4523	$C_{10}H_{14}O_2$	Slightly soluble	MS, 1H-NMR, IR	NA	mp: 185 º
Draft	3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one		166.22	Soluble	>95%	NA	
			Solid/cool mint-like aroma				
86		51200-86-3					

GROUP 5: MENTHOL AND STRUCTURALLY RELATED SUBSTANCES

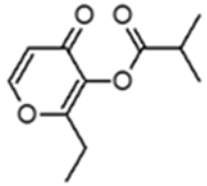
JECFA No. / Status / Session	Name / Chemical Name / Synonyms / CAS	FEMA / FLAVIS / CE	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2246	**Menthyl formate**	4509	$C_{11}H_{20}O_2$	Practically insoluble to insoluble	MS, IR	1.446-1.452	racemic mixture
Draft	2-isopropyl-5-methylcyclohexyl formate		184.28 Clear colorless liquid/Sweet minty aroma	Sparingly soluble 219-220°	>95%	0.933-0.939	
							
86		2230-90-2					
2247	**Menthyl propionate**	4510	$C_{13}H_{24}O_2$	Practically insoluble to insoluble	MS	1.444-1.449	
Draft	2-isopropyl-5-methylcyclohexyl propionate		212.34 Clear colorless liquid/fruity cool aroma	Sparingly soluble 246-247 º	>95%	0.918-0.923	
							
86		86014-82-6					

2248	**l-Menthyl butyrate**	4524	$C_{14}H_{26}O_2$	Practically insoluble to insoluble	MS	1.445-1.450	
Draft	2-isopropyl-5-methylcyclohexyl butyrate		226.36 clear colorless liquid/tropical cool aroma	Sparingly soluble 262-263 º	>95%	0.912-0.915	
							
86		68366-64-3					
2249	**dl-Isomenthol**	4729	$C_{10}H_{20}O$	Practically insoluble to insoluble	MS, 1H-NMR, IR	NA	mp: 82 º; racemic mixture
Draft	5-methyl-2-(propan-2-yl)cyclohexan-1-ol		156.27	Sparingly soluble	>95%	NA	
	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1-alpha,2-beta,5-beta)-(±)-; Menthol, trans-1,3,cis-1,4-(±)-; (±)-Isomenthol; Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1-alpha,2-beta,5-beta)-; Isomenthol		Solid/cool minty aroma				
	(1S, 2R, 5R) (+)-Isomenthol 						
	(1R, 2S, 5S) (-)-Isomenthol 						
86		3623-52-7					

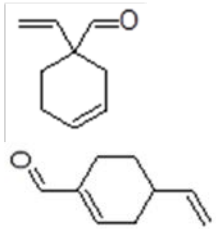
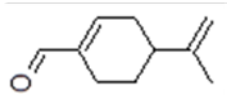
2250	**Dimenthyl glutarate**	4604	$C_{25}H_{44}O_4$	Practically insoluble to Insoluble	1H-NMR	NA	mp: Approx. 40 °
Draft	bis(2-isopropyl-5-methylcyclohexyl) glutarate	09.935	408.61	Very slightly soluble	>95%	NA	
	Pentanedioic acid, bis[5-methyl-2-(1-methylethyl)cyclohexyl] ester (9CI);Glutaric acid, di-(p-menth-3-yl) Ester		Amber amorphous or crystalline solid/Fresh minty aroma		< 1.0		
							
86		406179-71-3					
427	**Menthol**	2665	$C_{10}H_{20}O$	very soluble in alcohol and volatile oils; slightly soluble in water	IR	1.461	Melting Range: 41-44° ((-)-menthol); Nonvolatile Residue: =< 0.05%; Angular Rotation: -52° to -40° ((-)-menthol): -2° to +2° (dl-menthol)
	3-p-Menthanol						
Full			156.27		95%; sum of (+/-) isomers	0.901 (20°); 0.891 (30°)	
			colourless, hexagonal crystals, usually needle-like; fused masses or crystalline powder with a pleasant, peppermint-like odour				
		63		212° ((-)-isomer 216.5)			
	 l-menthol d-menthol						
51		89-78-1					

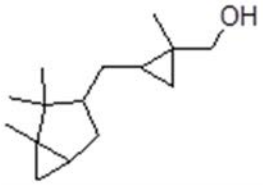
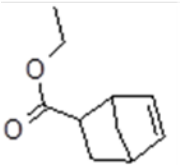
2251	**(±)2-[(2-p-Menthoxy)ethoxy]ethanol**	4718	$C_{14}H_{28}O_3$	Practically insoluble to insoluble	MS, 1H-NMR, IR	1.444 -1.484	(1R, 2S, 5R) (1S, 2R, 5S)
Draft	2-(2-((2-isopropyl-5-methylcyclohexyl)oxy)ethoxy)ethan-1-ol		244.37	Soluble	>95% (as a sum of the + and – isomers; racemic mixture)	0.947 - 0.987 (20°); 0.945 -0.985 (25°)	
	2-[2-(2-Isopropyl-5-methyl-cyclohexyloxy)-ethoxy]-ethanol		Colorless viscous liquid/Minty herbal aroma with fruity notes	97° (0.2 mmHg)	NA		
86		28804-53-7					

GROUP 6: MALTOL AND RELATED SUBSTANCES

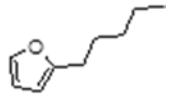
JECFA No. Status Session	Name Chemical Name Synonyms CAS	FEMA FLAVIS CE CAS	Chemical Formula M.W Physical form; Odour	Solubility Solubility in ethanol B.P. °	ID test Assay min % Acid value	R.I. (20°) S.G. (25°)	Other requirements Information required
2252	**Ethyl maltol isobutyrate**	4534	$C_{11}H_{14}O_4$	Practically insoluble to insoluble	MS, IR	1.480-1.496	Secondary component: 2-3% Ethyl maltol (No. 148
Draft	2-ethyl-4-oxo-4H-pyran-3-yl isobutyrate		210.23	Soluble	93-94%	1.132-1.138	
			Clear light yellow liquid/sweet fruity aroma	58-65 º (2 mm Hg)			
							
86		852997-28-5					

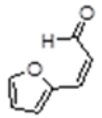
GROUP 7: ALICYCLIC PRIMARY ALCOHOLS, ALDEHYDES, ACIDS AND RELATED ESTERS

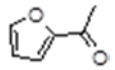
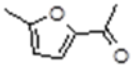
JECFA No.	Name	FEMA	Chemical Formula	Solubility Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W		Assay min %	S.G. (25°)	
	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		Information required
Session		CAS					
2253	**Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde** 1-vinylcyclohex-3-ene-1-carbaldehyde and 4-vinylcyclohex-1-ene-1-carbaldehyde	4783	$C_9H_{12}O$ 136.19 Liquid; Spicy herbal fruit aroma	Very slightly soluble Soluble 205 °	MS, 1H-NMR, 13C-NMR >95% (sum of mixture)	1.4870 - 1.4930 0.9565 - 09665	60%-70% 1-vinyl-3-cyclohexenecarbaldehyde and 25-35% 4-vinyl-1-cyclohexenecarbaldehyde
Draft							
86	1049017-63-1 1049017-68-6	1049017-63-1; 1049017-68-6					
973	**p-Mentha-1,8-dien-7-al** p-Mentha-1,8-dien-7-al	3557	$C_{10}H_{14}O$	Insoluble in water; soluble in alcohols and oils Miscible at room temperature	NMR	1.504-1.513 0.948-0.956	Safety evaluation not completed
Full			150.22		97%		
	Perilla aldehyde;Dihydrocuminic aldehyde;Perillaldehyde;4-Isopropenyl-1-cyclohexene-1-carboxaldehyde	11788	Pale, yellowish oily liquid; powerful, fatty-spicy, oily-herbaceous odour	104° (10 mm Hg)	3		
86		2111-75-3					

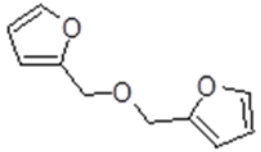
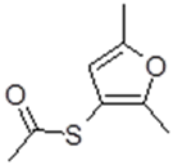
2254	**(1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol**	4776	$C_{15}H_{26}O$	very slightly soluble	MS, 1H-NMR, 13C-NMR	1.4820 - 1.4880	1S,2S 55% 1R,2R 40%
Draft	1-methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol		222.37	Soluble	>95%	0.941 - 0.951 (20°)	
	Javanol		Liquid; Strong musky, sweet, balsamic woody aroma reminiscent of sandalwood	268 °			
86		198404-98-7					
2255	**(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester**	4790	$C_{10}H_{14}O_2$	very slightly soluble	MS, 1H-NMR	1.448-1.488	Racemic mixture of R- and S-
Draft	ethyl bicyclo[2.2.1]hept-5-ene-2-carboxylate		166.09	Soluble	>95%	1.007-1.047 (20°)	
	Ethylbicyclo[2.2.1]hept-5-ene-2-carboxylate; 5-Norbornene-2-carboxylic acid, ethyl ester; 2,5-Endomethylene-3-cyclohexene carboxylic acid, ethyl ester		Clear liquid; Floral aroma with earthy fermented undertones	215-217 °			
86		10138-32-6					

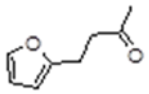
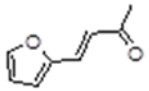
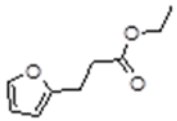
GROUP 8: FURAN SUBSTITUTED ALIPHATIC HYDROCARBONS, ALCOHOLS, ALDEHYDES, KETONES, CARBOXYLIC ACIDS AND RELATED ESTERS, SULFIDES, DISULFIDES AND ETHERS

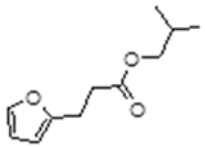
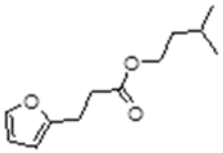
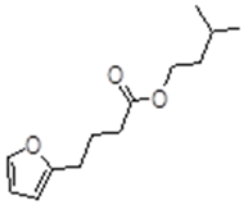
JECFA No.	Name	FEMA	Chemical Formula	Solubility Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W		Assay min %	S.G. (25°)	
	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		Information required
Session		CAS					
1491	**2-Pentylfuran**	3317	$C_9H_{14}O$	Slightly soluble in water	NMR	1.443-1.449	
Full	2-Pentylfuran	13.059	138.21	Soluble	99%	0.886-0.893	
	2-Amylfuran		Colourless liquid; Fruity aroma	58-60° (10 mm Hg)	1		
							
86		3777-69-3					
1492	**2-Heptylfuran**	3401	$C_{11}H_{18}O$	Insoluble in water	NMR	1.446-1.452	
Full	2-Heptylfuran	13.069	166.26	Soluble	99%	0.860-0.866	
			Colourless to yellowish liquid; Nutty, coffee-like aroma	209-210°	1		
							
86		3777-71-7					

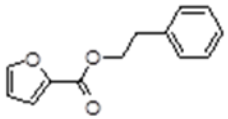
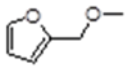
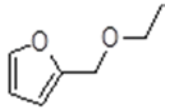
1493	**2-Decylfuran**	4090	$C_{14}H_{24}O$	Insoluble in water	NMR (13C)		mp: 30°
Full	2-Decylfuran	13.106	208.34 Colourless solid; Spicy, fatty aroma	Soluble	95%		
							
86		83469-85-6					
1494	**3-Methyl-2-(3-methylbut-2-enyl)-furan**	4174	$C_{10}H_{14}O$	Slightly soluble in water	MS	1.473-1.479	
Full	3-Methyl-2-(3-methylbut-2-en-**1**-yl)furan Rosefuran;2-(3-Methyl-2-butenyl)-3-methylfuran	13.148	150.22 Colourless liquid; Caramel aroma	Soluble 70° (11 mm Hg)	98% 1	0.998-1.004	
86		15186-51-3					
1497	**3-(2-Furyl)acrolein**	2494	$C_7H_6O_2$	Insoluble in water	NMR		mp: 49-52°
Full	3-(2-Furyl)prop-2-enal Furylacrolein;3-(2-Furyl)acrylaldehyde;3-(2-Furyl)-2-propenal;2-Furanacrolein	13.034	122.12 White or yellow needles; Cooked spicy-herb aroma	Soluble	97% 3		
							
86		623-30-3					

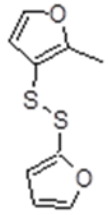
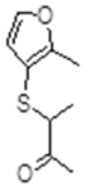
1499	**3-(5-Methyl-2-furyl)prop-2-enal**	4175	$C_8H_8O_2$	Slightly soluble in water	NMR (13C)	1.006-1.012
Full	3-(5-Methyl-2-furyl)prop-2-enal	13.150	136.15	Soluble	95%	0.998-1.004
	(5-Methylfuryl)acrolein;3-(5-Methyl-2-furanyl)-2-propenal;1-(5-Methyl-2-furanyl)-1-propen-3-al;5-Methyl-2-furanacrolein		Colourless liquid; Sweet spicy aroma	101° (5 mm Hg)	3	
86		5555-90-8				
1503	**2-Furyl methyl ketone**	3163	$C_6H_6O_2$	Very slightly soluble in water; Slightly soluble in propylene glycol, vegetable oils	IR	1.505-1.510
Full	2-Acetylfuran	13.054	110.11	Soluble	97%	1.102-1.107
	Acetylfuran;Methyl 2-furyl ketone		Yellow to brown liquid; Coffee-like aroma	67° (10 mm Hg)	1	
						
86		1192-62-7				
1504	2-Acetyl-5-methylfuran	3609	$C_7H_8O_2$	Slightly soluble in water; Soluble in corn oil	HNMR IR	1.511-1.517
Full	2-Acetyl-5-methylfuran	13.083	124.14	Soluble	99%	1.066-1.072 (20°)
	Methyl 5-methyl-2-furyl ketone;1-(5-Methyl-2-furyl) ethanone		Colourless liquid; Strong, nutty aroma	71-72° (8 mm Hg)	2	
						
86		1193-79-9				

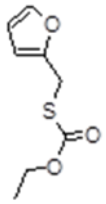
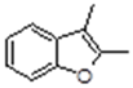
1522	**Difurfuryl ether**	3337	$C_{10}H_{10}O_3$	Insoluble in water	NMR	1.138-1.144
Full	Difurfuryl ether	13.061	178.19	Soluble	97%	1.506-1.512
	Furfuryl ether		Colourless to yellow liquid; Coffee-like, nutty aroma	88-89° (1 mm Hg)	1	
						
86		4437-22-3				
1523	**2,5-Dimethyl-3-furanthiol acetate**	4034	$C_8H_{10}O_2S$	Practically insoluble in water and hexane; Soluble in ether	HNMR IR MS	1.527-1.533
Full	2,5-Dimethyl-3-thioacetoxyfuran	13.116	170.23	Soluble	98%	1.137-1.143
	S-(2,5-Dimethyl-3-furyl) ethanethioate;Thioacetic acid S-(2,5-dimethyl-furan-3-yl) ester		Colourless liquid; Fruity floral aroma	230°	5	
						
86		55764-22-2				

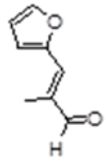
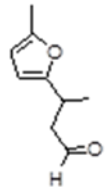
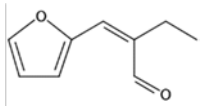
1510	**1-(2-Furyl)butan-3-one**	4120	$C_8H_{10}O_2$	Slightly soluble in water	MS	mp: 37°
Full	1-(2-Furyl)butan-3-one 1-(2-Furyl)-3-butanone;4-(2-Furyl)-2-butanone;Furfurylacetone	13.138	138.17 Colourless solid; Spicy caramel aroma	Soluble	95%	
						
86		699-17-2				
1511	**4-(2-Furyl)-3-buten-2-one**	2495	$C_8H_8O_2$	Insoluble in water	NMR	mp: 37-40°
Full	4-(2-Furyl)but-3-en-2-one Furylidene acetone;Furfural acetone	13.044	136.15 Colourless needle crystals; Spicy aroma	Soluble	98% 1	
						
86		623-15-4				
1513	**Ethyl 3-(2-furyl)propanoate**	2435	$C_9H_{12}O_3$	Very slightly soluble in water	NMR	mp: 24-25°
Full	Ethyl 3(2-furyl)propionate Ethyl furfurylacetate;Ethyl furylpropionate;Ethyl 2-furanpropionate	13.022	168.19 Low melting solid, turning yellow on exposure to air; Fruity aroma	Soluble	95% 5	
						
86		10031-90-0				

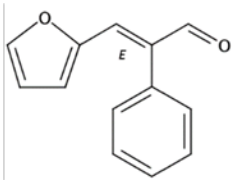
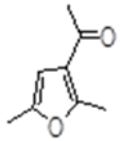
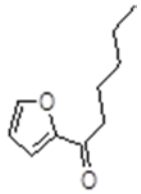
1514	**Isobutyl 3-(2-furan)propionate**	2198	$C_{11}H_{16}O_3$	Very slightly soluble in water	NMR	1.531-1.537
Full	2-Methylpropyl 3-(2-furyl)propanoate Isobutyl 2-furanpropionate;Isobutyl furylpropionate;Isobutyl 3-(2-furyl)propanoate	13.024	196.25 Colourless to pale, straw-yellow liquid; Fruity, winey, brandy-like aroma	Soluble 105° (3 mm Hg)	96% 5	1.007-1.013
86		105-01-1				
1515	**Isoamyl 3-(2-furan)propionate**	2071	$C_{12}H_{18}O_3$	Insoluble in water	NMR	1.549-1.557
Full	3-Methylbutyl 3-(2-furan)propanoate 2-Isoamyl furfurylacetate;3-Methylbutyl 3(2-furyl)propionate;Isoamyl 2-furanpropionate	13.023	210.27 Colourless to pale yellow liquid; Sweet, green, slightly floral aroma	Soluble 258°	96% 5	0.987-0.993
86		7779-67-1				
1516	**Isoamyl 4-(2-furan)butyrate**	2070	$C_{13}H_{20}O_3$	Insoluble in water	NMR	1.551-1.555
Full	3-Methylbutyl 4-(2-furan)butanoate Isopentyl 2-furanbutyrate;alpha-Isoamyl furfurylpropionate;3-Methylbutyl 2-furanbutyrate	13.021	224.3 Pale yellowish liquid; Sweet-buttery, fruity and caramel-like aroma	Soluble 263-265°	95% 5	0.975-0.981
86		7779-66-0				

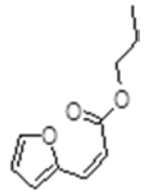
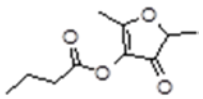
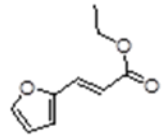
1517	**Phenethyl 2-furoate**	2865	$C_{13}H_{12}O_3$	Insoluble in water; Soluble in oils	NMR	1.585-1.593
Full	Phenethyl 2-furoate	13.006	216.24	Soluble	96%	1.136-1.142
	2-Phenylethyl 2-furoate;Phenylethyl 2-furoate		Colourless liquid; Warm, fruity-caramel, slightly earthy, oily aroma	275°	5	
						
86		7149-32-8				
1520	**Furfuryl methyl ether**	3159	$C_6H_8O_2$	Insoluble in water; Soluble in ether	NMR	1.454-1.460
Full	Furfuryl methyl ether	13.052	112.13	Soluble	99%	1.013-1.019
	Methyl furfuryl ether		Clear to yellow liquid; Airy, roasted coffee aroma	134-135°	1	
						
86		13679-46-4				
1521	**Ethyl furfuryl ether**	4114	$C_7H_{10}O_2$	Slightly soluble in water	MS	1.449-1.455
Full	Ethyl furfuryl ether	13.123	126.15	Soluble	95%	0.982-0.988
	Furfuryl ethyl ether		Colourless liquid; Sweet, spicy aroma	150°	1	
						
86		6270-56-0				

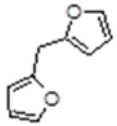
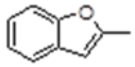
1524	**Furfuryl 2-methyl-3-furyl disulfide**	4119	$C_{10}H_{10}O_2S_2$	Slightly soluble in water; Soluble in pentane, diethyl ether	HNMR IR	1.581-1.587	SC: 6-7% Di-(2-methyl-3-furyl) disulfide
Full	Furfuryl 2-methyl-3-furyl disulfide 3-[2-Furanylmethyl)dithio]-2-methylfuran;(2-Methyl-3-furyl)furfuryl disulfide;2-Methyl-3-[(2-furanylmethyl)-dithio]furan;3-(Furfuryldithio)-2-ethylfuran		226.32	Soluble	90%	1.277-1.283	
			Colourless liquid; Strong, sulfurous aroma	294°	3		
							
86		109537-55-5					
1525	**3-[(2-Methyl-3-furyl)thio]-2-butanone**	4056	$C_9H_{12}O_2S$	Soluble in ethyl acetate, triacetin, Practically insoluble in water	HNMR MS	1.510-1.516	
Full	3-((2-Methyl-3-furyl)thio)-2-butanone 3-[(2-Methyl-3-furanyl)sulfanyl]-2-butanone;3-(2-Methyl-3-furylthio)-2-butanone;3-[(2-Methyl-3-furyl)sulfanyl]-2-butanone	13.190	184.25	Soluble	99%	1.104-1.110	
			Colourless liquid; Spicy, floral aroma	70° (0.75 mm Hg)	1		
							
86		61295-44-1					

1526	**O-Ethyl S-(2-furylmethyl)thiocarbonate**	4043	$C_8H_{10}O_3S$	Practically insoluble in water; Soluble in diether ether, ethyl acetate	HNMR IR MS	1.504-1.510	
Full	O-Ethyl S-(2-furylmethyl)thiocarbonate		186.23	Soluble	99%	1.167-1.173	
	O-Ethyl S-(2-furanylmethyl)thiocarbonate;Ethoxy carbonyl furfurylthiol;O-Ethyl S-(furan-2-ylmethyl) thiocarbonate		Colourless liquid; Spicy, floral aroma	130-135°	5		
							
86		376595-42-5					
1495	**2,3-Dimethylbenzofuran**	3535	$C_{10}H_{10}O$	Insoluble in water, Soluble in fats	NMR	1.554-1.563	
Full	2,3-Dimethylbenzofuran	13.074	146.19 Clear to yellow liquid; Nutty spicy aroma	Soluble 96-98° (15 mm Hg)	97% 1	1.031-1.037	
							
86		3782-00-1					
1496	**2,4-Difurfurylfuran**	4095	$C_{14}H_{12}O_3$	Insoluble in water	NMR (13C)		mp: 153°
Full	2,4-Difurfurylfuran	13.107	228.24 Colourless solid; Floral, fruity aroma	Soluble	95%		
86		64280-32-6					

1498	**2-Methyl-3(2-furyl)acrolein**	2704	$C_8H_8O_2$	Insoluble in water; Soluble in oils	NMR	1.567-1.573
Full	3-(2-Furyl)-2-methylprop-2-enal	13.046	136.15	Solube	96%	1.097-1.103
	2-Methyl-3-(2-furyl)propenal;alpha-Methylfurylacroleine;Furfurylidene-2-propanal		Pale yellowish liquid; Mild, warm, cinnamon-like aroma	225°	3	
						
86		874-66-8				
1500	**3-(5-Methyl-2-furyl)-butanal**	3307	$C_9H_{12}O_2$	Insoluble in water; Soluble in oils	NMR	1.575-1.581
Full	3-(5-Methyl-2-furyl) butanal	13.058	152.19	Soluble	98%	1.006-1.012
	3-(5-Methyl-2-furyl)-butyraldehyde		Colourless liquid; Vegetable, fruity aroma	88-91° (12 mm Hg)	3	
						
86		31704-80-0				
1501	**2-Furfurylidenebutyraldehyde**	2492	$C_9H_{10}O_2$	Insoluble in water; Soluble in oils	NMR	1.570-1.576
Full	Furfurylidene-2-butanal	13.043	150.18	Soluble	98%	1.057-1.063
	3(2-Furyl)-2-ethylacrolein;2-Ethyl-3(2-furyl)-2-propenal;2-Ethyl-3(2-furyl)acrolein		Pale yellowish liquid; Mild, warm, vegetable-like aroma	240°	3	
						
86		770-27-4				

1502	**2-Phenyl-3-(2-furyl)prop-2-enal**	3586	$C_{13}H_{10}O_2$	Insoluble in water	NMR		mp: 56-57°
Full	3-(2-Furyl)-2-phenylprop-2-enal	13.137	198.22	Soluble	99%		
	2-Furfurylidenephenylacetaldehyde		White solid; Berry aroma				
							
86		65545-81-5					
1506	**3-Acetyl-2,5-dimethylfuran**	3391	$C_8H_{10}O_2$	Slightly soluble in water; Soluble in propylene glycol, most fixed oils	NMR	1.488-1.490	
Full	3-Acetyl-2,5-dimethylfuran	13.066	138.17	Soluble	99%	1.037-1.039	
	2,5-Dimethyl-3-acetylfuran		Clear to yellow liquid; Powerful, slightly roasted, nutty aroma	83° (11 mm Hg)	1		
							
86		10599-70-9					
1512	**Pentyl 2-furyl ketone**	3418	$C_{10}H_{14}O_2$	Slightly soluble in water	NMR	1.490-1.496	
Full	2-Hexanoylfuran	13.070	166.22	Soluble	99%	0.992-0.998	
	2-Furyl pentyl ketone		Colourless to yellow liquid; Apricot, peach-like aroma	65-67° (0.5 mm Hg)	1		
							
86		14360-50-0					

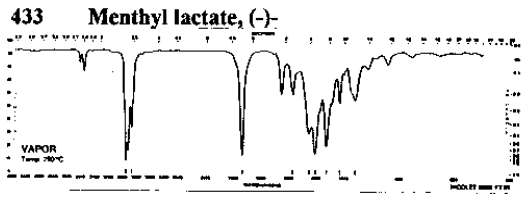
1518	**Propyl 2-furanacrylate**	2945	$C_{10}H_{12}O_3$	Insoluble in water	NMR		
Full	Propyl 3(2-furyl)prop-2-enoate	13.047	180.2	Soluble	97%	1.071-1.077 (20°)	
	2-Propenoic acid, 3-(2-furanyl)-, propyl ester;Propyl 3-(2-furyl)acrylate		Colourless to pale yellow liquid; Light strawberry, pear-like aroma	119° (7 mm Hg)	5		
							
86		623-22-3					
1519	**2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate**	3970	$C_{10}H_{14}O_4$	Insoluble in water	NMR	1.467-1.473	SC: 1-3% 4-Hydroxy-2,5-dimethyl-3(2H)-furanone and 1-3% Butyric acid
Full	4-Butyroxy-2,5-dimethyl-3(2H)-furanone		198.22	Soluble	93%	1.095-1.103	
			Colourless to pale yellow liquid; Spicy, sweet aroma	287°	5		
							
86		114099-96-6					
2103	**(E)-Ethyl 3-(2-furyl)acrylate**	4541	$C_9H_{10}O_3$	Practically insoluble to insoluble in water	MS	1.542-1.548	
Full	Ethyl (2E)-3-(furan-2-yl)prop-2-enoate		166.17	Soluble	95%	1.090-1.096	
	Ethyl(E)-3-(2-furyl)-2-propenoate		Viscous liquid; Sweet aroma	230-233°			
							
86		53282-12-5					

2104	**di-2-Furylmethane**	4540	$C_9H_8O_2$	Practically insoluble to insoluble in water	MS	1.501-1.507 1.097-1.103
Full	2,2'-Methanediyldifuran		148.16	Soluble	95%	(20°)
	di-2-Furyl methane		Colourless clear liquid; Rich roasted aroma	194-195°		
						
86		1197-40-6				
2105	**2-Methylbenzofuran**	4543	C_9H_8O	Practically insoluble to insoluble in water	MS	1.548-1.560
Full	2-Methyl-1-benzofuran		132.16	Soluble	95%	1.052-1.057
	2-Methyl benzo(b)furan		Colourless liquid; Burnt phenolic aroma	197-198°		
						
86		4265-25-2				

REVISIONS TO EXISTING FLAVOUR SPECIFICATIONS

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / COE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
433	*/*-menthyl */*-lactate	3748	C13H24O3		IR		Melting point c.25°
Full	(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl (2S)-2-hydroxypropanoate		228.33 colourless liquid or white crystalline solid with a weak chamomile or tobacco odour	142° (5 mmHg)	97% 2		
86		61597-98-6					
619	l-malic acid						Melting point: 100°; Fumaric Acid: max. 1.0%; Heavy Metals: max 10 ppm; Maleic acid: max. 0.05%; Residue on Ignition: max 0.1 %; Water Insoluble Matter: max. 0.1%
		2655	$C_4H_6O_5$	soluble in water and alcohol; 1 gm in 0.8 ml water	IR		
Full	2-Hydroxybutanedioic acid		134.09	1 g in 1.4 ml alcohol	99%		
	Hydroxysuccinic acid;2-Hydroxybutanedioic acid	17	White crystalline powder, granules, or needles; Acid taste; odourless or having a very faint caramellic acrid odour and a tart aciduous taste				
86							
2123	Glutamyl-valyl-glycine	4709	C12H21N3O6	Soluble in water	MS, HNMR		m.p.: 200-204º (decomposition)
Full	(2S)-2-Amino-5-({(2S)-1-[(carboxymethyl)amino]-3-methyl-1-oxobutan-2-yl}amino)-5-oxopentanoic acid		303.31	Practically insoluble	>95%		
	L-gamma-glutamyl-L-valyl-glycine	38837-70-6	Solid off-white powder; Light savoury almost yeastlike aroma				
86							

SPECTRA OF CERTAIN FLAVOURING AGENTS



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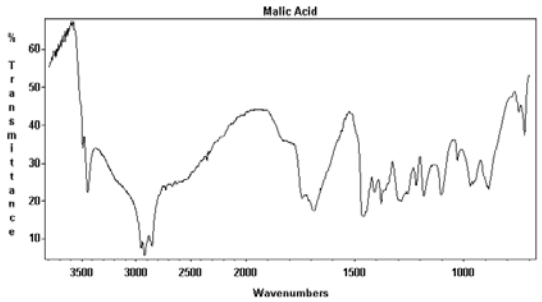

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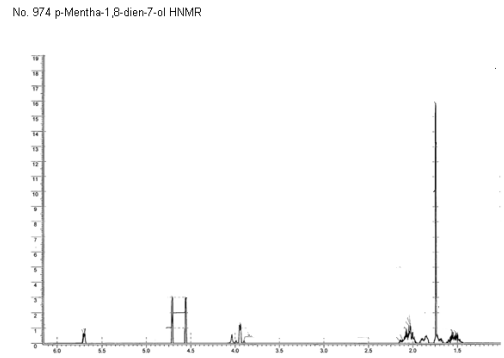

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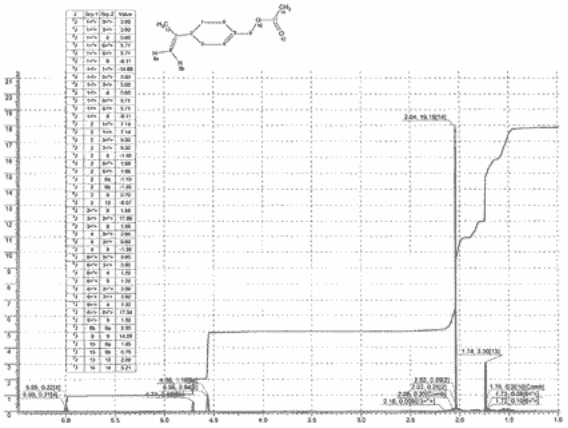

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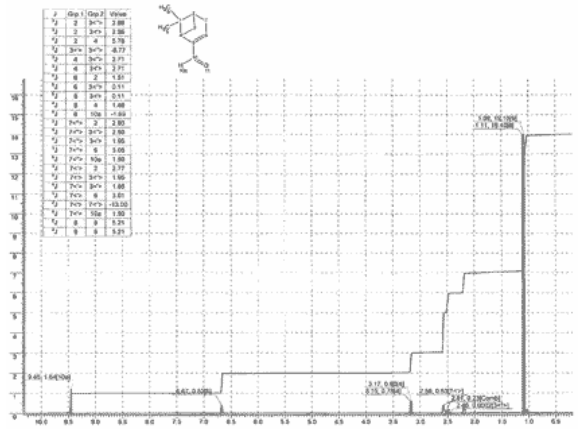

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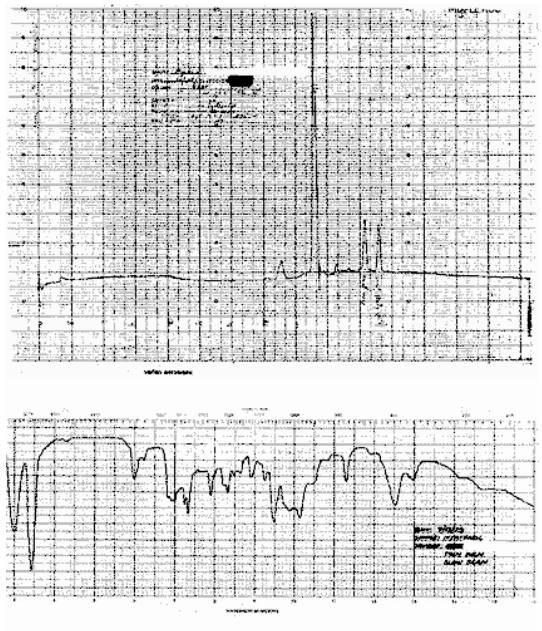

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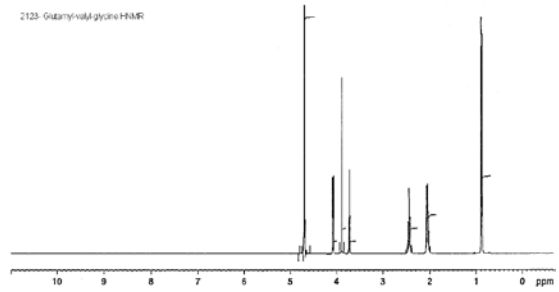

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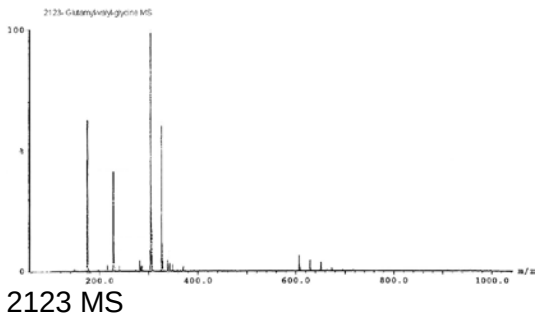

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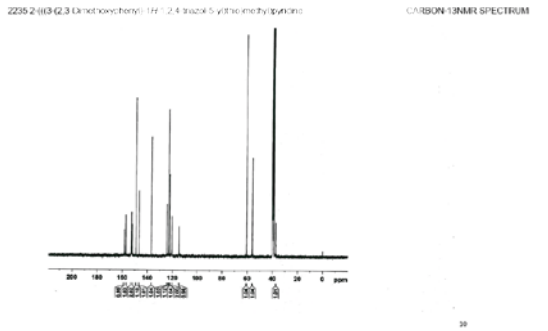
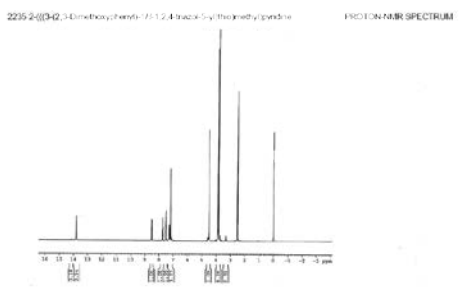


2235 C13 NMR



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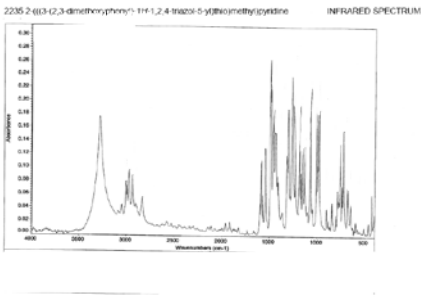

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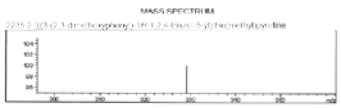

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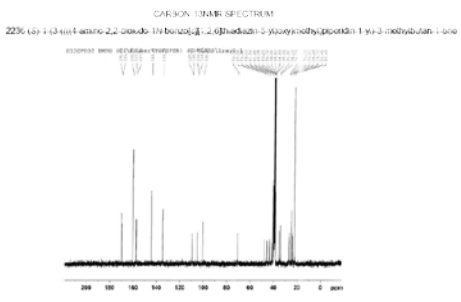

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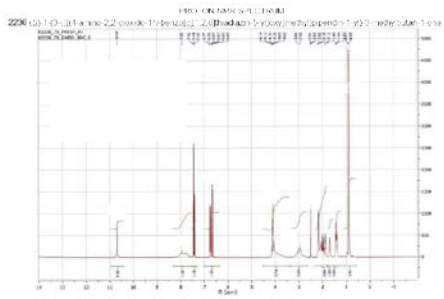

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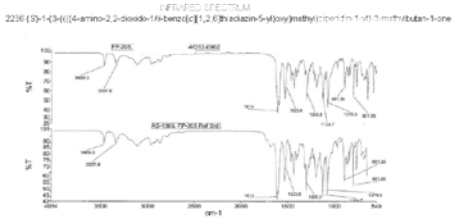

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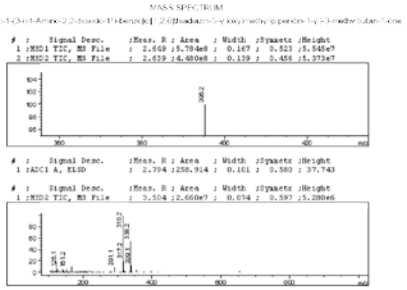

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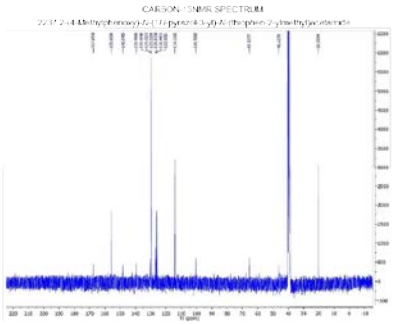
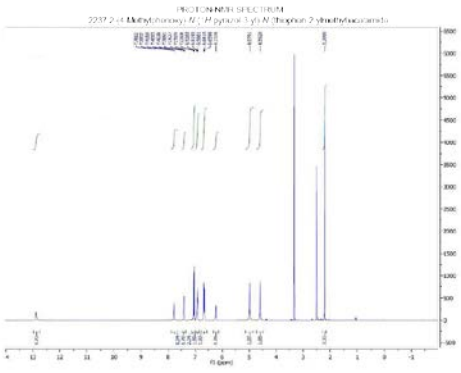


2237 C13NMR



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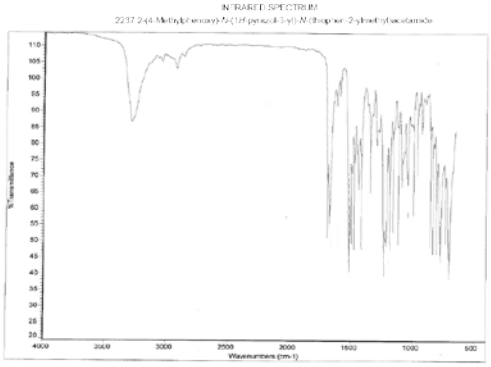

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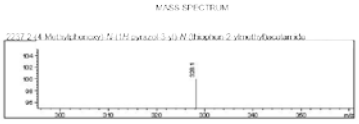

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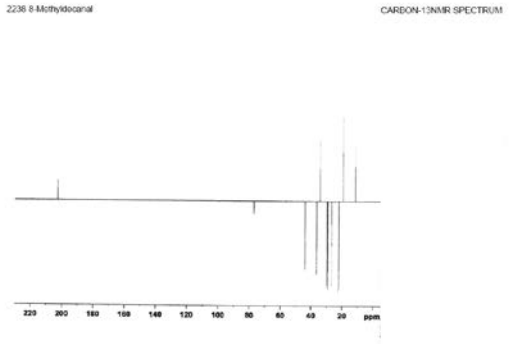
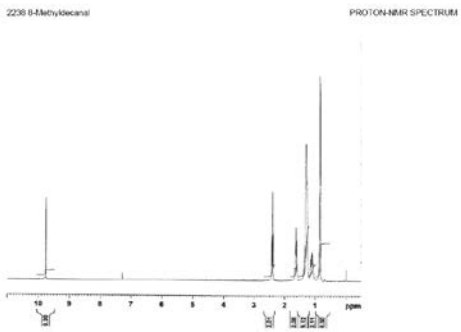


2238 C13NMR



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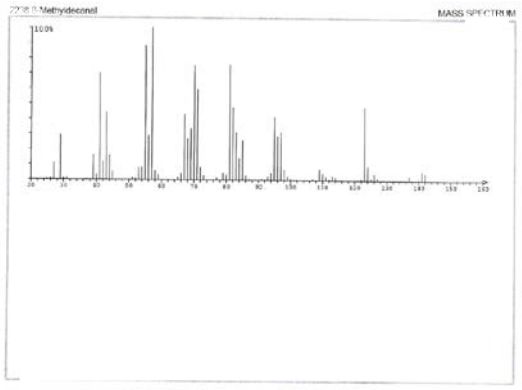

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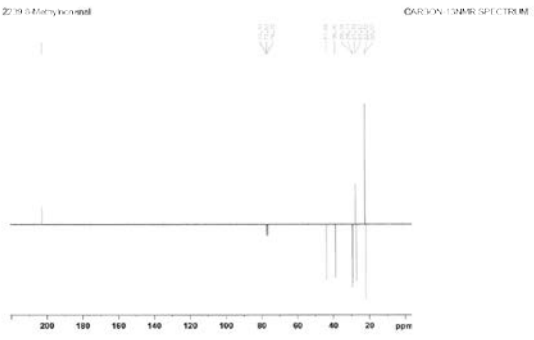

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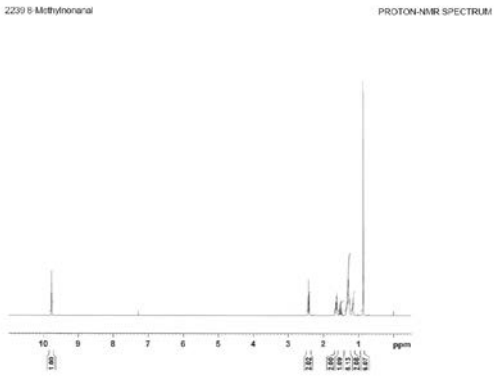

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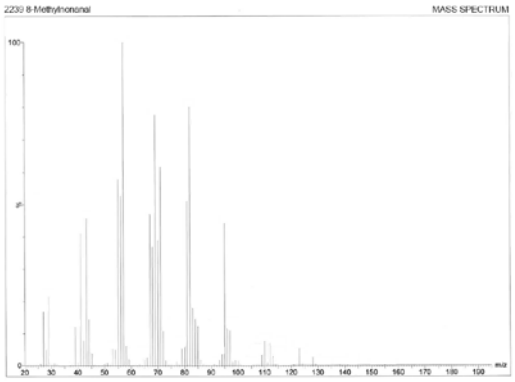

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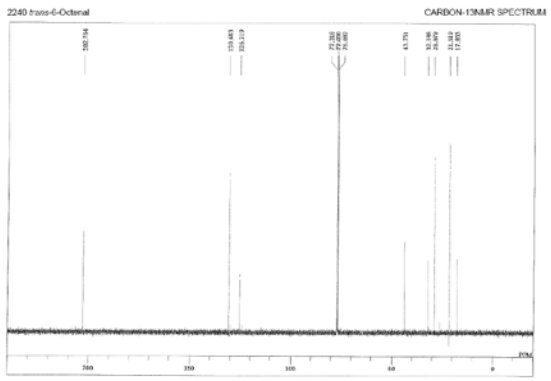
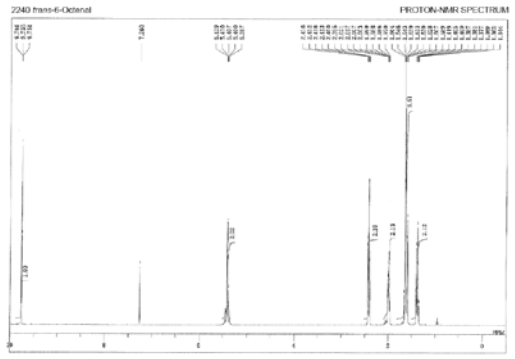
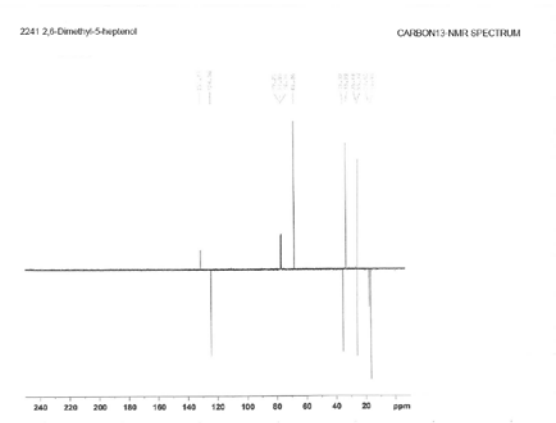


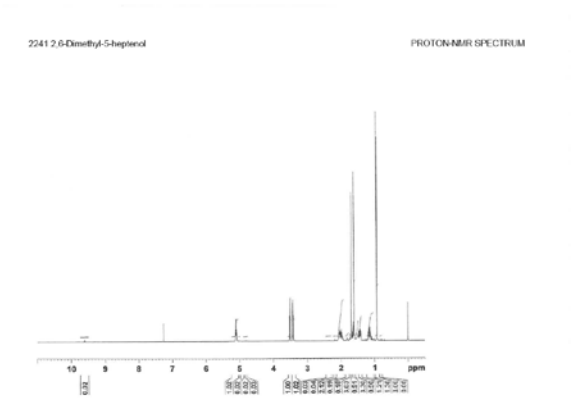
2240 C13NMR



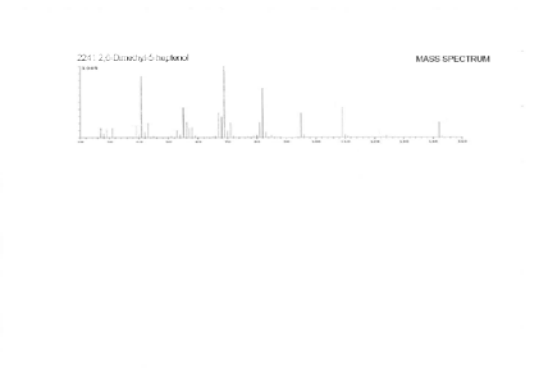
2240 HNMR



2241 C13NMR



2241 HNMR



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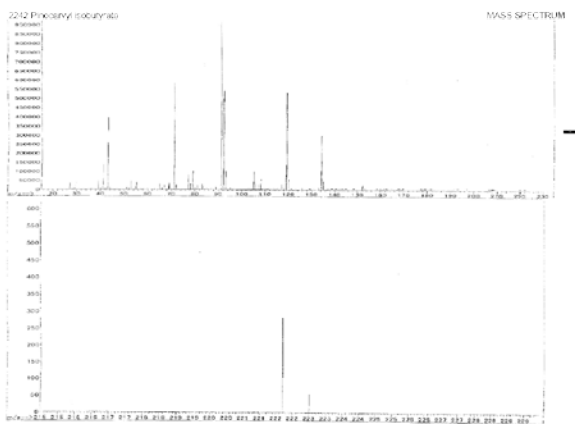
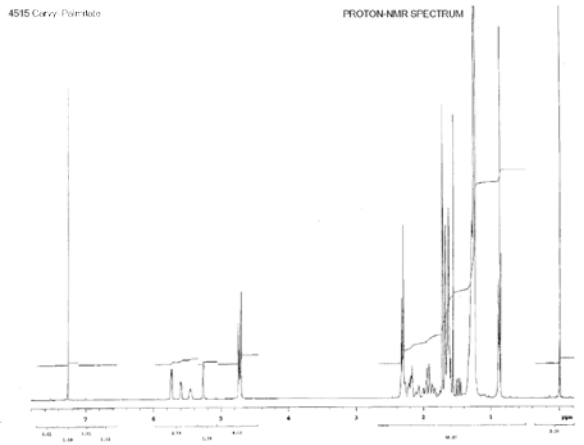
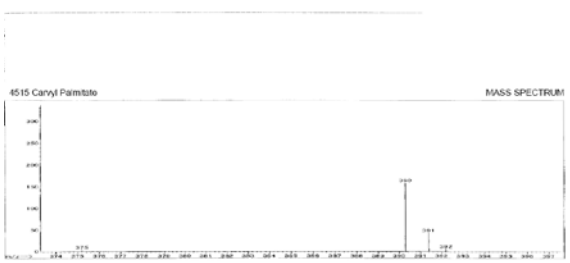


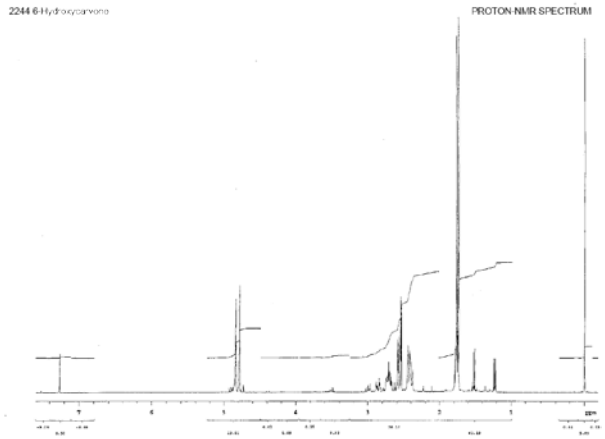
2242 MS



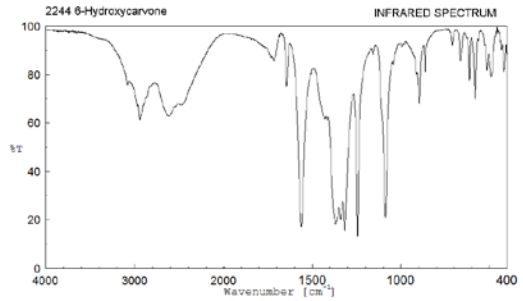
2243 HNMR



2243 MS



2244 HNMR



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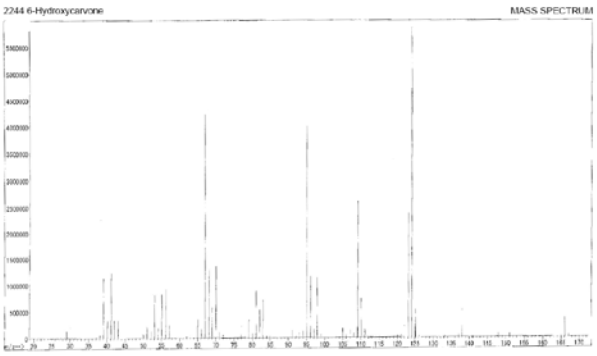

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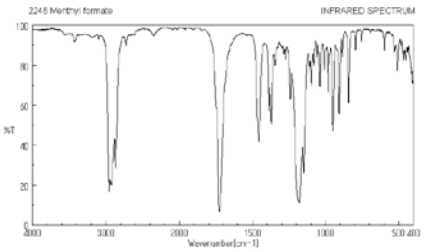

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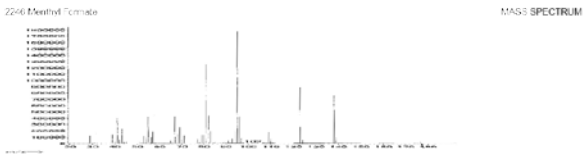

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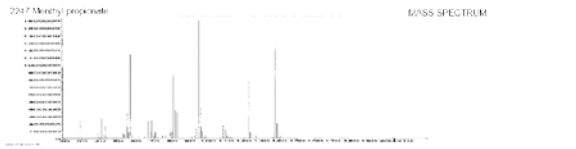

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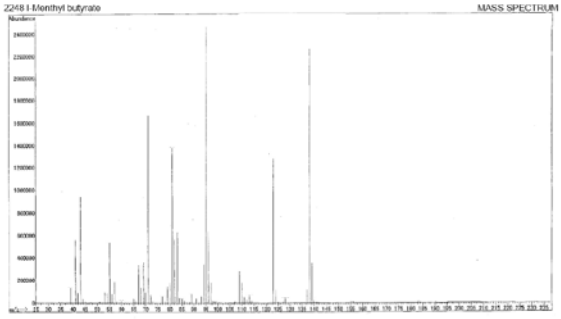

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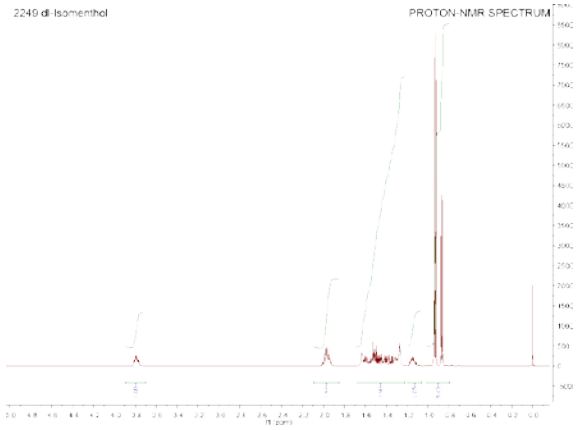
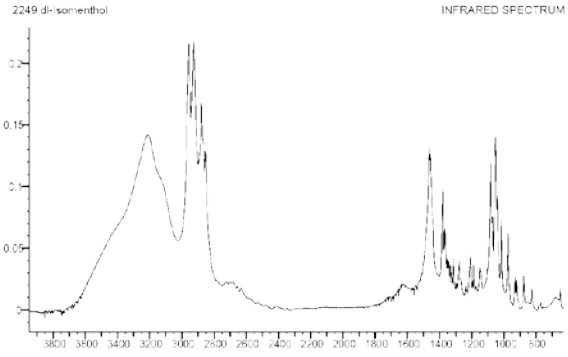


2249 HNMR



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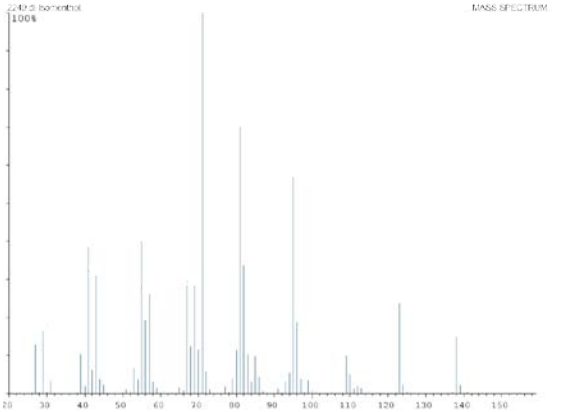

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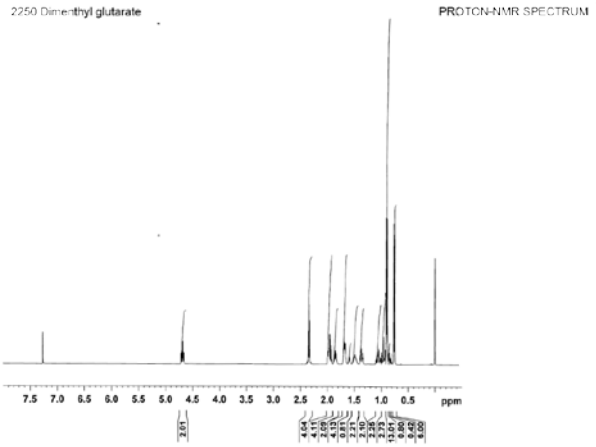
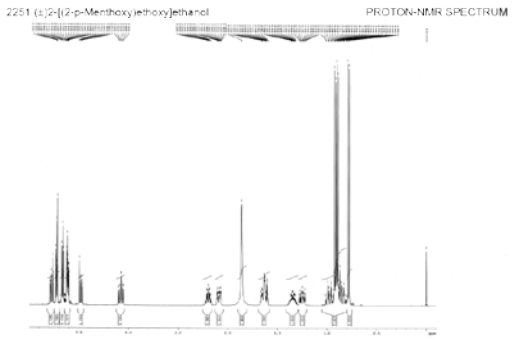


2250 HNMR



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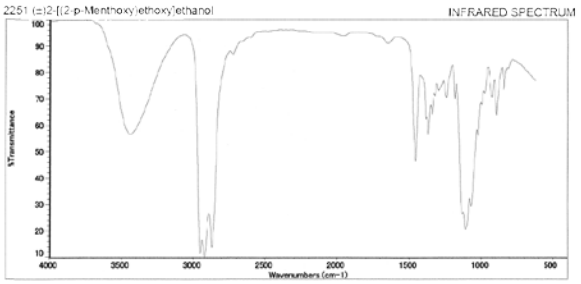

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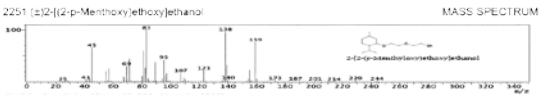

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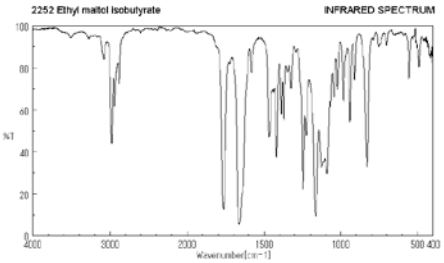

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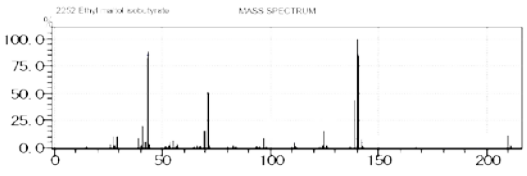

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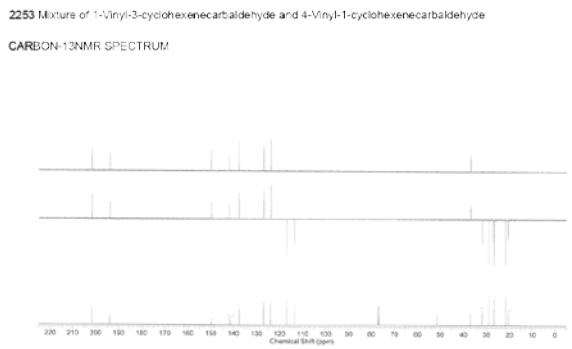

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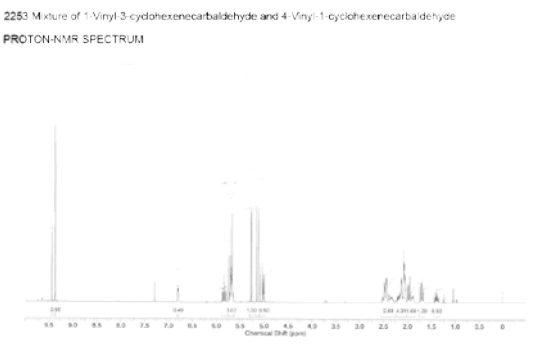

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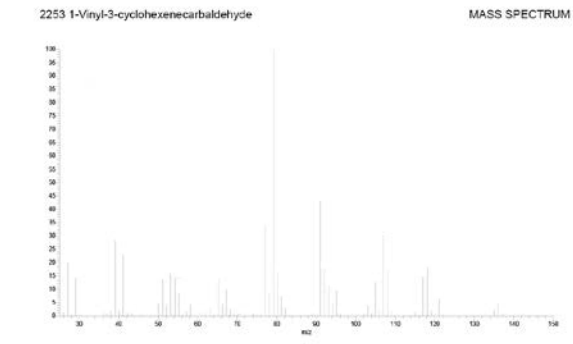

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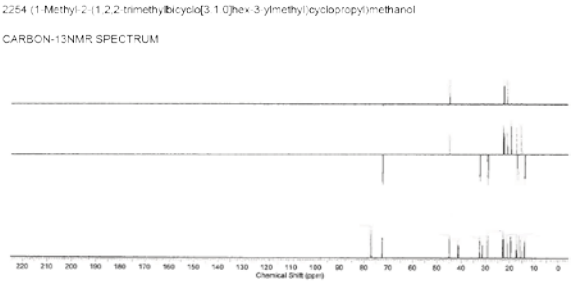

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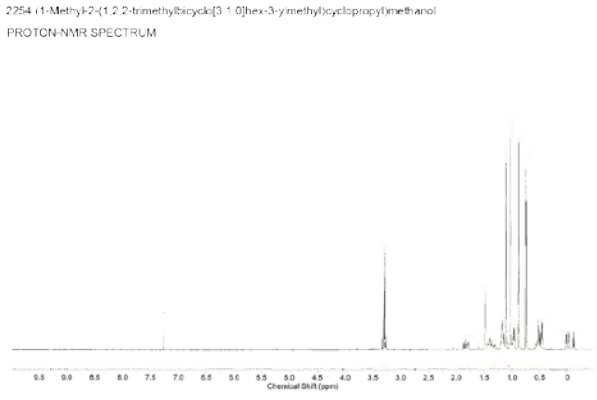

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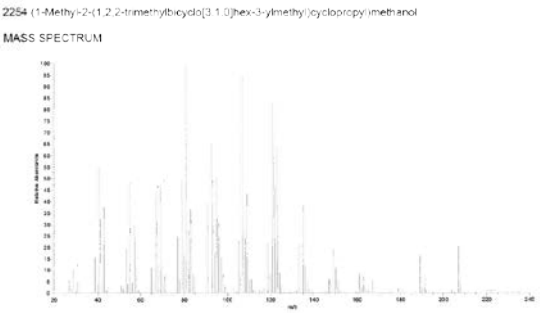

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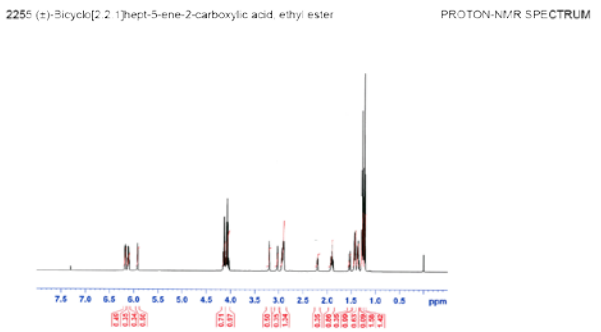

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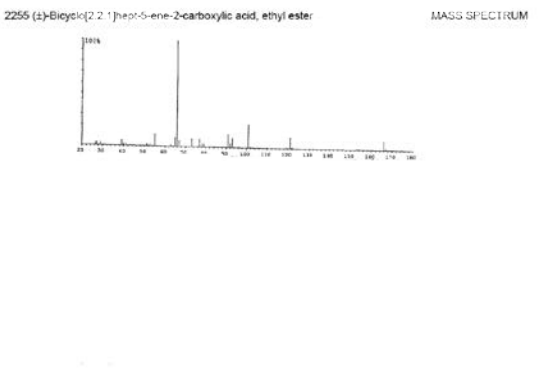
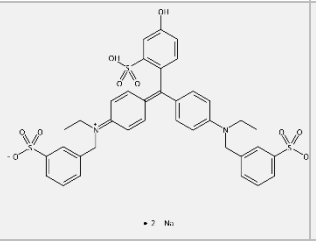


2255 MS

CORRIGENDUM

The following requests for corrections, reported to the JECFA secretariats, were evaluated by the 86th JECFA meeting and found to be necessary. These corrections, however, will only be made in the electronic versions and in the on-line database.

Food additive	Original text	New text	Additional information
Calcium disodium ethylenediaminetetraacetate (INS 385) Monograph 1 (2006)	CAS No. 662-33-9	CAS No. 62-33-9	Transcription error
Chlorophyllins, copper complexes sodium and potassium salts (INS 141(ii)) Monograph 5 (2008) Test for "Free ionisable copper"	Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil….	Accurately weigh about 1 g of the sample and **mix** in 20 ml of arachid oil….	Correction
Curcumin (INS: 100(i)) Monograph 1 (2006)	The criteria for several residual solvents are listed under the heading "Residual solvents" (see Fig. 1).	Acetone: Not more than 30 mg/kg Hexane: Not more than 25 mg/kg Methanol: Not more than 50 mg/kg Ethanol: Not more than 50 mg/kg Isopropanol: Not more than 50 mg/kg Ethyl acetate: Not more than 50 mg/kg	Improves readability It was unclear whether the criterion "Not more than 50 mg/kg" extended to methanol, ethanol, isopropanol and ethyl acetate.
Ethyl acetoacetate ethyleneglycol ketal JECFA No: 1969 JECFA 73 (2010)	CAS No. 1648615	CAS No. 6413-10-1	Transcription error
Ethyl 2-methyl pentanoate JECFA No: 214 JECFA 55 (2000)	CAS No. 28959-02-6	CAS No. 39255-32-8	Wrong CAS number
***cis*-3-Hexen-1-ol JECFA No.: 315 JECFA 51 (1998)**	98.0% (sum of (*Z*) and (*E*) isomers, =<92.0% (*Z*))	98.0% (sum of (*Z*) and (*E*) isomers, =>92.0% (*Z*))	Transcription error
Monosodium L-glutamate (INS: 621) Monograph 1 (2006)	CAS No. 142-47-2	CAS No. 6106-04-3	Wrong CAS number
Myrcene JECFA No.: 1327 JECFA 63 (2004)	Specific gravity: 0.789–1.793	Specific gravity: 0.789–0.793	Transcription error

Food additive	Original text	New text	Additional information
Polyoxyethylene (20) sorbitan monostearat (Polysorbate 60) (INS 435) Monograph 16 (2014)	CAS No. 9005-07-6	CAS No. 9005-67-8	Wrong CAS number
Sodium aluminium silicate (INS 554) Monograph 20 (2017)	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on dried basis".	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on **ignited** basis".	Transcription error
Silicon dioxide, amorphous (INS 551) Monograph 20 (2017)	CAS No. 112696-00-8 (hydrated silica)	CAS No. 112926-00-8 (hydrated silica)	Transcription error
	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface a**d**sorbed water.	Transcription error
Sodium thiosulfate (INS 539) Monograph 1 (2006)	CAS No. 7772-98-7	CAS No. 10102-17-7	CAS No. 7772-98-7 refers to the anhydrous form. The specifications in the monograph refer to the pentahydrate form.
Brown HT and its aluminium lake (FAO JECFA Monographs 19, 82nd meeting, 2016)	Text in the Table 1 "Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry"	See Table 1, below	
Fast Green FCF (FAO JECFA Monographs 19, 82nd meeting, 2016)	Chemical structure in Table 1 "Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry"		

CAS: Chemical Abstracts Service; INS: International Numbering System for Food Additives; No.: number
Bolding and underlining for clarity only. This formatting will not be shown in the online database.

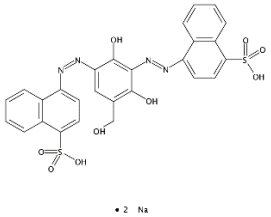
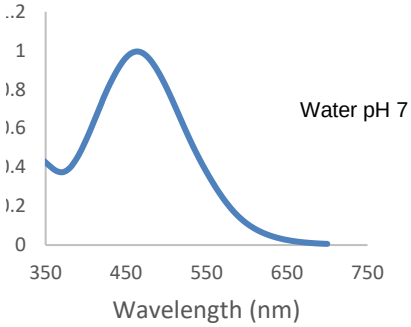
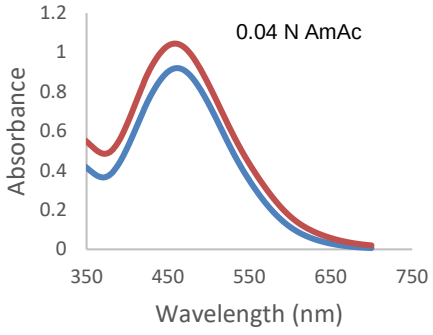
The criteria for several residual solvents are listed under the heading “Residual solvents” (see Fig. 1)

Figure1: Residual solvent criteria for curcumin as displayed in Monograph 1, 2006

Residual solvents (Vol. 4)	Acetone:	Not more than 30 mg/kg
	Hexane:	Not more than 25 mg/kg
	Methanol:	}
	Ethanol:	Not more than 50 mg/kg
	Isopropanol:	
	Ethyl acetate:	

Table 1

Replacement of the text for the spectrophotometric data for Brown HT and its aluminium lake originally published in “Table 1. Values for synthetic colours for use in performing tests for Colouring Matters Content by Spectrophotometry” (FAO JECFA Monographs 19, 82nd meeting, 2016)

JECFA name	Sample weight	Structure	Spectral data	Visible absorption spectrum
Brown HT	245.6 mg		Water, pH 7 λ_{max} = 464 A = 0.9957 Spec abs = 403 a = 40.3 Water λ_{max} = 464 A = 0.9804 Spec abs = 397 a = 39.7 0.04 N AmAc λ_{max} = 461 A = 0.9206 Spec abs = 373 a = 37.3	
Brown HT Aluminium Lake	53.3 mg		Straight colour (blue) 0.04 N AmAc λ_{max} = 461 A = 0.9206 Lake (red) 0.04 N AmAc λ_{max} = 458 A = 1.0451	

ANNEX I: SUMMARY OF RECOMMENDATIONS FROM THE 86th JECFA

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Eighty-sixth meeting
Geneva, 12–21 June 2018

SUMMARY AND CONCLUSIONS
Issued 3 July 2018

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Geneva, Switzerland, from 12 to 21 June 2018. The purpose of the meeting was to evaluate certain food additives (including flavouring agents).

Dr A. Mattia, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, served as Chairperson, and Dr. Richard Cantrill, Canada, served as Vice-Chairperson.

Dr M. Lipp, Office for Food Safety, Food and Agriculture Organization of the United Nations, and Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, served as Joint Secretaries.

The present meeting was the eighty-sixth in a series of similar meetings. The tasks before the Committee were (a) to undertake safety evaluations of certain food additives (including flavouring agents); and (b) to review and prepare specifications for certain food additives (including flavouring agents).

The Committee evaluated the safety of eight food additives, revised the specifications for 19 other food additives (including 16 modified starches), evaluated 69 flavouring agents according to the revised Procedure for the Safety Evaluation of Flavouring Agents and revised the specifications for three flavouring agents.

The report of the meeting will be published in the WHO Technical Report Series. Its presentation will be similar to that of previous reports – namely, general considerations, comments on specific substances and recommendations for future work. An annex will include detailed tables (similar to the tables in this report) summarizing the main conclusions of the Committee in terms of acceptable daily intakes and other toxicological, dietary exposure and safety recommendations. Information on the specifications for the identity and purity of certain food additives (including flavouring agents) examined by the Committee will also be included.

The participants in the meeting are listed in Annex 1. Items of a general nature that the Committee would like to disseminate quickly are included in Annex 2. Future work and recommendations are listed in Annex 3.

Toxicological and dietary exposure monographs on most of the substances that were considered will be published in WHO Food Additives Series No. 77. New and revised specifications for the identity and purity of the compounds will be published in FAO JECFA Monographs 22.

More information on the work of JECFA is available at:

http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/

and

http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Anionic methacrylate copolymer (AMC)	N, T[a]	**The Committee was unable to complete the evaluation of AMC.** While the copolymer itself is not of health concern, genotoxicity concerns remains for the residual monomer methacrylic acid. The specifications were made tentative pending the completion of the safety evaluation of AMC.
Basic methacrylate copolymer (BMC)	N	**The Committee established an ADI "not specified" for basic methacrylate copolymer.** The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification. The NOAELs for BMC ranged from 750-2000 mg/kg bw per day which were the highest doses tested. The Committee evaluated exposure to BMC for the copolymer and its monomers (n-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate). Estimated exposures to BMC range from 3.0 to 135 mg/kg bw per day. The total monomeric content of BMC is less than 0.3%. The Committee concluded that the toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.
Erythrosine	R[b]	**The Committee concluded that the new data that have become available since the previous evaluation of erythrosine do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw.** The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to erythrosine for all age groups do not present a health concern.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Indigotine	R[b]	The Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies and **concluded that there are no reasons to revise the ADI and confirmed the previous ADI of 0–5 mg/kg bw**. The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper bound of the ADI of 0–5 mg/kg bw. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.
Lutein	R[c,d]	Free lutein, lutein esters and free zeaxanthin including *meso*-zeaxanthin are biochemically and toxicologically equivalent. At the present meeting the Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from *Tagetes erecta*, synthetic zeaxanthin and *meso*-zeaxanthin. Free lutein, lutein esters and free zeaxanthin and *meso*-zeaxanthin are substances of low toxicity for which no adverse effects have been observed in a broad range of toxicological studies in laboratory animals and clinical studies in humans. Based on the absence of toxicity in a wide range of studies, **the Committee established a group ADI "not specified" for lutein from *Tagetes* erecta, lutein esters from *Tagetes* erecta and zeaxanthin (synthetic).** M*eso*-zeaxanthin was not included in this group ADI, as specifications are not currently available. **The group ADI of 0-2 mg/kg bw for lutein from *Tagetes erecta* and zeaxanthin (synthetic) was withdrawn.**
Neutral methacrylate copolymer (NMC)	N, T	**The Committee established an ADI "not specified" for NMC. The ADI "not specified" was made temporary because the specifications are tentative**. The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes. The NOAELs for NMC ranged from 454–2000 mg/kg bw per day, and these were the highest doses tested. The Committee evaluated exposure to NMC for the copolymer and its monomers (methyl methacrylate and ethyl acrylate). Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day. The total monomeric content of NMC is less than 0.01%. Toxicological data on the residual monomers do not

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		give rise to concerns when taking into account the low dietary exposures.
Sorbitol syrup	-	Sorbitol syrup (INS 420(ii)) is currently included in the Codex General Standard for Food Additives (GSFA) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. The Committee was therefore requested to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances, and advise on the need for a separate evaluation of sorbitol syrup or if the ADI "not specified" for sorbitol is also applicable for sorbitol syrup. Based on the similarity of the chemical constituents of sorbitol syrup to the previously evaluated sorbitol, maltitol syrup and polyglycitol syrup, **the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI "not specified" for sorbitol syrup.**
Spirulina extract	N, T	**The Committee established a temporary ADI "not specified" for spirulina extract.** The ADI was based on the absence of toxicity in repeated-dose animal studies with spirulina extract and dried spirulina. **The ADI "not specified" was made temporary due to the tentative nature of the specifications**. Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour based on the Budget method and exposure to spirulina extract and dried spirulina from other dietary sources, including food ingredients, dietary supplements, and coatings of food supplements was 190 mg/kg bw for adults (60 kg/person) and 650 mg/kg bw for a child (15 kg/person). The Committee concluded that this dietary exposure does not present a health concern.

- : no specifications prepared; N: new specifications; R: existing specifications revised; T: tentative specifications

[a] The specifications were made tentative pending the completion of the safety evaluation of AMC.

[b] At the current meeting, high-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

[c] The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

[d] At the current meeting, the identity test for melting range was deleted, the identity tests for carotenoids and spectrophotometry were updated, the test for propylene glycol was incorporated verbatim and the previous reference removed, and the method of assay was updated.

Food additives considered for specifications only

Food additive	Specifications
Cassia gum	R[a]
Citric and fatty acid esters of glycerol	R, T[b]
Glycerol ester of wood rosin	R[c]
Modified starches	R[d], T

R: existing specifications revised; T: tentative specifications

[a] The Committee, at its current meeting, received analytical methods and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with n-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified. The specifications were revised and the tentative status was removed.

[b] The Committee did not receive a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in its specifications monograph. The Committee noted further that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform. Specifications were revised and made tentative pending the availability of data. Specifications will be withdrawn if suitable information is not provided by December 2019.

[c] The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species namely *Pinus halepensis* and *Pinus brutia* as source materials. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. Since the specifications monograph for GEWR does not contain an assay, the Committee recommended that the JECFA Secretariat be notified upon the development and validation of an appropriate assay. The existing specifications were revised.

[d] The Committee reviewed data on the method of manufacture, identity, and purity of all 16 modified starches. Based on the information received, and available information the Committee noted that:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered the application of a read-across approach to be appropriate for the toxicological evaluation of these substances.
- All 16 modified starches had been assigned an ADI of “not specified”.
- All modified starches can be additionally bleached or fragmented; therefore revision in the specifications of bleached or fragmented starches would imply the revision of all 16 monographs;
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals impurities content and microbiological considerations). Revision of those common specifications would affect all 16 monographs;
- As a result of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, potentially unavailable, and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.
- Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs should be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture, and the corresponding diversity of impurities. The Committee recommended that all modified starches be included in a

modular monograph titled 'Modified Starches' that contains common requirements [General specifications for modified starches] consisting of specifications that apply to all 16 modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The Committee drafted a new modular specifications monograph titled "Modified starches" consisting of an explanatory introduction, "General specifications for modified starches," and eight annexes. The new modular specifications monograph for modified starches is printed in FAO Monograph 22, and will replace the 16 existing individual specifications for modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

The specification for lead included in the General specifications be decreased from 2 mg/kg to 0.2 mg/kg. The limit of lead for starch sodium octenylsuccinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added.

Flavouring agents evaluated by the revised Procedure for the Safety Evaluation of Flavouring Agents

A. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde	2253	N	No safety concern
p-Mentha-1,8-dien-7-ol	974	N	No safety concern
p-Mentha-1,8-dien-7-yl acetate	975	N	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	N	No safety concern
Myrtenol	981	N	No safety concern
Myrtenyl acetate	982	M	No safety concern
Structural class II			
(1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	N	No safety concern
Structural class III			
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	N	No safety concern
Flavouring agent excluded at Step 1 of the Procedure			
p-Mentha-1,8-dien-7-al (Perillaldehyde)	973	M	Genotoxicity data for *p*-mentha-1,8-dien-7-al raise concerns for potential genotoxicity

N: new specifications

M: existing specifications maintained;

B. Carvone and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Pinocarvyl isobutyrate	2242	N	No safety concern
Carvyl palmitate	2243	N	No safety concern
Structural class III			
6-Hydroxycarvone	2244	N	No safety concern
Flavouring agents not evaluated according to the revised Procedure			
(+)-Carvone	380.1	M	The Committee did not re-evaluate (+)-carvone (No. 380.1) according to the revised Procedure given the lack of information on the oral exposure from all sources and the need to review the ADI. A review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.
(-)-Carvone	380.2	M	The Committee did not re-evaluate (-)-carvone (No. 380.2) according to the revised Procedure given the lack of information on the oral exposure from all sources and the lack of toxicological data.

M: existing specifications maintained; N: new specifications

C. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-Pentylfuran	1491	M[a]	No safety concern
2-Heptylfuran	1492	M[a]	No safety concern
2-Decylfuran	1493	M[a]	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	M[a]	No safety concern

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
2,3-Dimethylbenzofuran	1495	M[a]	No safety concern
2,4-Difurfurylfuran	1496	M[a]	No safety concern
3-(2-Furyl)acrolein	1497	M[a]	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	M[a]	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	M[a]	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	M[a]	No safety concern
2-Furfurylidene-butyraldehyde	1501	M[a]	No safety concern
2-Phenyl-3-(2-furyl)prop-2-enal	1502	M[a]	No safety concern
2-Furyl methyl ketone	1503	M[a]	No safety concern
2-Acetyl-5-methylfuran	1504	M[a]	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	M[a]	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	M[a]	No safety concern
2-Butyrylfuran	1507	M[a]	No safety concern
(2-Furyl)-2-propanone	1508	M[a]	No safety concern
2-Pentanoylfuran	1509	M[a]	No safety concern
1-(2-Furyl)butan-3-one	1510	M[a]	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	M[a]	No safety concern
Pentyl 2-furyl ketone	1512	M[a]	No safety concern
Ethyl 3-(2-furyl)propanoate	1513	M[a]	No safety concern
Isobutyl 3-(2-furan)propionate	1514	M[a]	No safety concern
Isoamyl 3-(2-furan)propionate	1515	M[a]	No safety concern
Isoamyl 3-(2-furan)butyrate	1516	M[a]	No safety concern
Phenethyl 2-furoate	1517	M[a]	No safety concern
Propyl 2-furanacrylate	1518	M[a]	No safety concern
2,5-Dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate	1519	M[a]	No safety concern
Furfuryl methyl ether	1520	M[a]	No safety concern
Ethyl furfuryl ether	1521	M[a]	No safety concern
Difurfuryl ether	1522	M[a]	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	M[a]	No safety concern
Furfuryl 2-methyl-3-furyl disulfide	1524	M[a]	No safety concern
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	M[a]	No safety concern
O-Ethyl S-(2-furylmethyl)thiocarbonate	1526	M[a]	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	M[a]	No safety concern
di-2-Furylmethane	2104	M[a]	No safety concern
2-Methylbenzofuran	2105	M[a]	No safety concern

M: existing specifications maintained

[a] The text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full

D. Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
trans-6-Octenal	2240	N	No safety concern
2,6-Dimethyl-5-heptenol	2241	N	No safety concern

N: new specifications

E. Maltol and related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Maltol	1480	M	No safety concern[a]
Structural class III			
Ethyl maltol isobutyrate	2252	N	No safety concern

M: existing specifications maintained

N: new specifications

[a] The previously established ADI for maltol was withdrawn by the Committee.

F. Menthol and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Menthyl formate	2246	N	No safety concern
Menthyl propionate	2247	N	No safety concern
l-Menthyl butyrate	2248	N	No safety concern
dl-Isomenthol	2249	N	No safety concern
Dimenthyl glutarate	2250	N	No safety concern
Menthol	427	M	No safety concern[a]
Structural class III			
(±)-2-[(2-*p*-Menthoxy)ethoxy]ethanol	2251	N	No safety concern

M: existing specifications maintained

N: new specifications

[a] The ADI of menthol of 0–4 mg/kg bw established at the fifty-first meeting was maintained.

G. Miscellaneous nitrogen-containing substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-(((3-(2,3-Dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	N	No safety concern
S)-1-(3-(((4-Amino-2,2-dioxido-1*H*-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	N	No safety concern
2-(4-Methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl)acetamide	2237	N	No safety concern

N: new specifications

H. Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
8-Methyldecanal	2238	N	No safety concern
8-Methylnonanal	2239	N	No safety concern

N: new specifications

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications
L-menthyl lactate	433	R[a]
L-malic acid	619	R[b]
Glutamyl-valyl-glycine	2123	R[c]

[a] The CAS number was changed from 59259-38-0 to 61597-98-6 and the name to L-menthyl L-lactate.

[b] The specification for specific rotation were removed

[c] The melting point range was revised

ANNEX 2. GENERAL INFORMATION

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ANNEX 3. FUTURE WORK AND RECOMMENDATIONS

SPECIFIC FOOD ADDITIVES (OTHER THAN FLAVOURING AGENTS)

Anionic Methacrylate Copolymer

The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid. Further studies to clarify the in vivo carcinogenic potential are required.

Citric and fatty acid esters of glycerol

The specifications of CITREM were made tentative, requiring a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.
The Committee noted that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform.
Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by **December 2019**.

Neutral Methacrylate Copolymer

The Committee noted that there was no data submitted for a suitable method of assay. Tentative specifications for NMC were prepared and made tentative requiring a suitable validated method of assay.

Spirulina extract

The Committee received limited analytical data on *spirulina* extract. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested by **December 2019**:

- Full compositional characterization of commercial products in both liquid and powder forms.
- Full compositional characterization of the aqueous extract before formulation/standardization.
- Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data).
- Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

Modified starches

The Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in Hydroxypropyl starch (INS 1440) and Hydroxypropyl distarch phosphate (INS 1442) in order to consider lowering this limit.

The Committee requests suitable microbiological acceptance criteria and supporting data for all modified starches.

Table 1. The annexes and the modified starches to which they apply along with required information:

ANNEX	Modification	Starches to which it applies	Information required
1	Minor fragmentation	INS 1400: Dextrin roasted starch; INS 1401: Acid treated starch; INS 1402: Alkaline treated starch; INS 1405: Enzyme-treated starch All modified starches that are additionally fragmented.	A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes.
2	Bleaching	INS 1403: Bleached starch All modified starches if additionally bleached.	Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s).
3	Esterification and/or crosslinking with phosphorus containing compounds	INS 1410: Monostarch phosphate; INS 1412: Distarch phosphate; INS 1413: Phosphated distarch phosphate; INS 1414: Acetylated distarch phosphate; INS 1442: Hydroxypropyl distarch phosphate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches.
4	Acetylation	INS 1420: Starch acetate; INS 1414: Acetylated distarch phosphate; INS 1422: Acetylated distarch adipate; INS 1451: Acetylated oxidized starch	Currently no additional information required.
5	Oxidation	INS 1404: Oxidized starch; INS 1451: Acetylated oxidized starch	A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method.
6	Esterification with octenyl succinic anhydride	INS 1450: Starch sodium octenyl succinate	Currently no additional information required.
7	Etherification with propylene epoxide	INS 1440: Hydroxypropyl starch; INS 1442: Hydroxypropyl distarch phosphate	A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of Hydroxypropyl starch using the method
8	Crosslinking with adipic anhydride	INS 1422: Acetylated distarch adipate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches. Levels of free adipic acid in at least 5 representative batches

FLAVOURING AGENTS

Carvone and structurally related substances

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within three years.

For (-)-carvone (No. 380.2), the Committee concluded that toxicological data on (-)-carvone are necessary. Also, data are needed for an exposure assessment for the oral exposure to (-)-carvone from all sources

Maltol and related substances

The Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol (No. 1480) during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting.

The Committee withdrew the ADI for maltol. The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the ADI.

The ADI for ethyl maltol was maintained.

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

86th Meeting 2018

This document contains food additive specification monographs, analytical methods, and other information prepared at the eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 12–21 June 2018. The specification monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additives are of the quality required for use in food or in processing and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.

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