



COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

84th Meeting 2017



Food and Agriculture
Organization of the
United Nations



World Health
Organization

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SPECIFICATIONS**

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Table of Contents

LIST OF PARTICIPANTS.....	VII
INTRODUCTION.....	11
SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES.....	12
BRILLIANT BLUE FCF	13
β -CAROTENE-RICH EXTRACT FROM <i>DUNALIELLA SALINA</i>	18
FAST GREEN FCF	22
GUM GHATTI	27
JAGUA (GENIPIN-GLYCINE) BLUE (TENTATIVE)	30
METATARTARIC ACID (TENTATIVE).....	38
MICROCRYSTALLINE CELLULOSE.....	41
SILICON DIOXIDE, AMORPHOUS.....	44
SODIUM ALUMINIUM SILICATE.....	47
STEVIOL GLYCOSIDES FROM <i>STEVIA REBAUDIANA</i> BERTONI.....	50
SUCROSE ESTERS OF FATTY ACIDS.....	70
TAMARIND SEED POLYSACCHARIDE.....	78
YEAST EXTRACTS CONTAINING MANNOPROTEINS (TENTATIVE).....	83
ANALYTICAL METHODS.....	88
TOTAL COLOURING MATTERS CONTENT (VOLUME 4).....	88
ERRATA: CAROB BEAN GUM	91
CORRIGENDUM.....	95
ANNEX I: SUMMARY OF RECOMMENDATIONS FROM THE 84 th JECFA.....	97
FOOD ADDITIVES CONSIDERED FOR SPECIFICATIONS ONLY.....	97
FOOD ADDITIVES EVALUATED TOXICOLOGICALLY AND ASSESSED FOR DIETARY EXPOSURE.....	98
ANNEX 2. GENERAL INFORMATION	103
INFORMATION REQUIREMENTS FOR SUBMISSIONS ON PRODUCTS DERIVED FROM NATURAL SOURCES	103
ANNEX 3. FUTURE WORK AND RECOMMENDATIONS.....	105

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 84th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome, 6–15 June 2017. 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.

Brilliant Blue FCF (R)
β-Carotene-rich extract from *Dunaliella Salina* (N)
Fast Green FCF (R)
Gum ghatti (R)
Jagua (Genipin–Glycine) Blue (N,T)
Metatartaric acid (T)
Microcrystalline cellulose (R)
Silicon dioxide, amorphous (R)
Sodium aluminium silicate (R)
Steviol glycosides (R)
Sucrose esters of fatty acids (R)
Tamarind seed polysaccharide (N)
Yeast extracts containing mannoproteins (N,T)

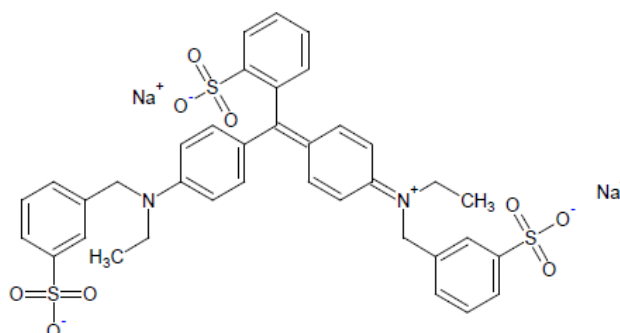
In the specifications monographs that have been assigned a tentative status (T), there is information on the outstanding data and a timeline by which this information should be submitted to the FAO JECFA Secretariat.

BRILLIANT BLUE FCF

Prepared at the 84th JECFA and published in JECFA Monograph 20 (2017) superseding specifications prepared at the 28th JECFA (1984) and published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-6 mg/kg bw was established at the 84th JECFA (2017).

SYNONYMS	INS No. 133, CI Food Blue 2, CI (1975) No. 42090, FD&C Blue No. 1
DEFINITION	<p>Consists essentially of disodium 3-[<i>N</i>-ethyl-<i>N</i>-[4-[[4-[<i>N</i>-ethyl-<i>N</i>-(3-sulfobenzyl)amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadiene-1-ylidene]ammoniomethyl]benzenesulfonate and its isomers together with subsidiary colouring matters, as well as sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by condensing 2-formylbenzenesulfonic acid with a mixture of 3-[(<i>N</i>-ethyl-<i>N</i>-phenylamino)methyl]benzenesulfonic acid and its 2- and 4-isomers to form the leuco base precursor. Oxidation of the leuco base precursor with either chromium or manganese containing compounds produces the dye, which is purified and isolated as the disodium salt.</p> <p>May be converted to the corresponding aluminium lake in which case only the requirements in the <i>General Specifications for Aluminium Lakes of Colouring Matters</i> apply.</p>
Chemical names	<p>Disodium 3-[<i>N</i>-ethyl-<i>N</i>-[4-[[4-[<i>N</i>-ethyl-<i>N</i>-(3-sulfobenzyl)amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadiene-1-ylidene]ammoniomethyl]benzenesulfonate;</p> <p><i>N</i>-ethyl-<i>N</i>-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium inner salt, disodium salt;</p> <p>Disodium;2-[[4-[ethyl-[(3-sulfonatophenyl)methyl]amino]phenyl]-[4-[ethyl-[(3-sulfonatophenyl)methyl]azaniumylidene]cyclohexa-2,5-dien-1-ylidene]methyl]benzenesulfonate;</p> <p>Disodium α-(4-(<i>N</i>-ethyl-3-sulfonatobenzylamino)phenyl)-α-(4-(<i>N</i>-ethyl-3-sulfonatobenzylamino)cyclohexa-2,5-dienylidene)toluene-2-sulfonate</p>
C.A.S. number	3844-45-9
Chemical formula	C ₃₇ H ₃₄ N ₂ Na ₂ O ₉ S ₃

Structural formula



Formula weight	792.86
Assay	Not less than 85% total colouring matters
DESCRIPTION	Blue powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Soluble in water; slightly soluble in ethanol
<u>Spectrophotometry</u> (Vol. 4)	Maximum wavelength approximately 629 nm Determine the UV-visible absorption spectrum of the sample solution dissolved in water.
PURITY	
<u>Loss on drying, chloride and sulfate as sodium salts</u> (Vol. 4)	Not more than 15% as total amount Determine chloride as sodium chloride, sulfate as sodium sulfate, and water content (loss on drying at 135°) as described in Volume 4 (under "Specific Methods, Food Colours").
<u>Water insoluble matter</u> (Vol. 4)	Not more than 0.2%
<u>Subsidiary colouring matters</u>	Not more than 6% See description under TESTS
<u>Organic compounds other than colouring matters</u>	Not more than 1.5%, sum of 2-, 3- and 4-formylbenzenesulfonic acids

Not more than 0.3% 3-[[*N*-ethyl-*N*-(4-sulfophenyl)amino]methyl]-benzene-sulfonic acid
See description under TESTS

Leuco base (Vol. 4) Not more than 5%
Weigh accurately 130±5 mg sample and proceed as directed under *Leuco Base in Sulfonated Triarylmethane Colours* (Vol. 4)
Absorptivity (a) = 164 L/(g·cm) at 629 nm
Ratio = 0.971

Un sulfonated primary aromatic amines (Vol. 4) Not more than 0.01% calculated as aniline

Ether extractable 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”).

Chromium (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”).

Manganese (Vol. 4) Not more than 100 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”).

TESTS

PURITY TESTS

Subsidiary colouring matters Determine subsidiary colouring matters content 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 ammonium acetate in water
- Eluent B: 0.05 M ammonium acetate in methanol
- Injection volume: 2 µl
- Column temperature: 40°
- Detector: UV-visible/PDA at 629 nm
- Flow rate: 0.2 mL/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	90	10
7	60	40
15	52	48
30	45	55
39	30	70
39.1	0	100
44	0	100
44.1	90	10
54	90	10

Standards:

Subsidiary colouring matters – synthesized materials
 Brilliant Blue FCF (C.A.S. No. 3844-45-9) – TCI, Cat. No. F0147 or equivalent (use if subsidiary colouring matter standards are not available)

Sample preparation:

Weigh accurately 500±5 mg sample and dissolve in 100 mL of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 629 nm. If Brilliant Blue FCF is used as the standard, calculate the ratio of the sum of all peaks not corresponding to Brilliant Blue FCF 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 above conditions for subsidiary colouring matters except:

Detector: UV-visible/PDA at 254 nm

Standards:

- 2-Formylbenzenesulfonic acid, sodium salt (C.A.S. No. 1008-72-6) – Sigma-Aldrich, Cat. No. 12050 or equivalent (use for quantitating the 2-, 3-, and 4-isomers)
- 3-[[N-ethyl-N-(4-sulfophenyl)amino]methyl]benzenesulfonic acid, calcium salt (C.A.S. No. 5363-53-1, acid form) – Wako, Cat. No. 031-23071 or equivalent

Sample preparation:

Weigh accurately 500 ± 5 mg sample and dissolve in 100 mL of water.

Calculations:

Construct the relevant standard curves. Calculate the sum of 2, 3, and 4-formylbenzenesulfonic acids as their sodium salts and 3-[[*N*-ethyl-*N*-(4-sulfophenyl)amino]methyl]benzenesulfonic acid as its sodium salt.

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 0.04 M aqueous ammonium acetate as the solvent:
absorptivity (a) = 164 L/(g·cm) and wavelength of maximum absorbance = 629 nm.

β-CAROTENE-RICH EXTRACT FROM *DUNALIELLA SALINA*

*Prepared at the 84th JECFA (2017) and published in JECFA Monograph 20 (2017). The 84th JECFA (2017) concluded that there was no health concern for the use of β-carotene-rich extract from *D. salina* when used as a food colour in accordance with the specifications established at 84th meeting. The Committee emphasized that this conclusion applies to the use of this extract as food colour, not as food or food supplement.*

SYNONYMS

Natural β-carotene, carotenes-natural; CI (1975) No. 75130; CI (1975) No. 40800 (β-Carotene)

DEFINITION

β-Carotene-Rich Extract from *Dunaliella salina* is obtained by extraction from strains of the algae *Dunaliella salina* (syn. *D. bardawil* and *D. Kone*) using the essential oil d-limonene. The extract is then prepared as a suspension in vegetable oil after removal of the essential oil. The main colouring principals are trans- and cis-isomers of β-carotene together with minor amounts of other carotenes including α-carotene, lutein, zeaxanthin and cryptoxanthin. The isomers of β-carotene account for approximately 90% of the carotenes in the product. Besides the colour pigments and vegetable oil, β-Carotene-Rich Extract from *Dunaliella salina* contains lipids and other fat-soluble components naturally occurring in the source material such as fatty acids typically found in food oils, long-chain alcohols, alkenes, and waxes. The main articles of commerce may be further blended with vegetable oil to standardize the colour content.

Class

Carotenoid

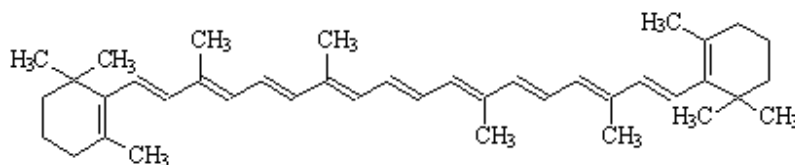
C.A.S. number

7235-40-7

Chemical formula

C₄₀H₅₆ (β-Carotene)

Structural formula



all-trans-β-Carotene

Formula weight	536.88 (β -Carotene)
Assay	Content of carotenes (calculated as β -carotene) is not less than declared See description under TESTS

DESCRIPTION Opaque, deep-red, viscous suspension

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; practically insoluble in ethanol; soluble in hexane

Spectrophotometry (Vol. 4) A cyclohexane solution of the sample shows maximum absorptions at 448 - 457 and 474 – 486 nm

Colour reaction A spot of a solution of the sample in toluene (about 400 μg /mL of β -carotene) on a filter paper turns blue 2-3 min after application of a spray or drop of 20% solution of antimony trichloride solution in toluene.

PURITY

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").

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 methods described in Vol. 4 (under "General Methods, Metallic Impurities").

d-Limonene Not more than 0.3% (w/w)
See description under TESTS

TESTS

PURITY TESTS

d-Limonene

Principle

Determination of essential oil limonene by gas chromatography (Vol. 4) under the following conditions:

Chromatography conditions

- Column: polyethylene glycol Agilent DB-WAX, or equivalent, 30m x 0.53mm x 1.0 microns
- Carrier Gas: High purity Nitrogen, 12kPa head pressure, 5:1 split or splitless
- Detector: Flame ionization detector, 200°
- Injection: 2.0 µL (split) or 0.5µl (splitless), 150°
- Temp. Program:
 - o Initial: 80°, 5.0 minute
 - o Rate: 10°/minute
 - o Final: 150°, 3 minutes
 - o Total run time: 15 minutes
- Quantitation:
 - o Calculate by: Area
 - o Method: Internal standard
 - o Curve Fit Type: Linear
- Internal standard solution: Prepare a 10.0 mg/mL solution of terpinene in n-hexane.
- Standard solutions: Prepare a range of solutions containing 0, 0.04, 0.1, 1.0 and 4.0 mg/mL of limonene with 0.4 mg/mL of terpinene in n-hexane.

Sample solution:

Accurately weigh a sample based on the expected quantity of limonene (1.0-2.5g) into a 25mL volumetric flask. Pipette 1 mL of the Internal standard solution into the flask and vortex 15 seconds. Add about 10mL dichloromethane and vortex 15 seconds. Make up to volume with dichloromethane. Mix thoroughly.

System Suitability Check

- Perform 2 injections of the blank. No limonene peak should be detected.
- Perform 5 injections of the standard solution containing 1.0 mg/mL of limonene and 0.4 mg/mL of terpinene in n-hexane. The coefficient of variation of 5 injections is <1.00%. Separation between the limonene and terpinene peaks should be not less than 0.5 min.

Calculations:

$$RF_1 = \frac{A_{istd} \times [STD]}{A_{std} \times [ISTD]}$$

$$[LIM] = \frac{A_{lim} \times DIL \times RF_1}{A_{istd} \times m}$$

$$[ISTD] = \frac{m_{istd}}{12.5}$$

Where:

- RF₁ = Response factor calculated from the calibration standard
 [STD] = Concentration of the standard as % w/w
 [ISTD] = Concentration internal standard as % w/w
 [LIM] = Concentration of limonene in the sample as % w/w
 A_{istd} = Area of the Internal standard peak
 A_{std} = Area of the limonene peak in the calibration standard
 A_{lim} = Area of the limonene peak in the sample
 DIL = Dilution factor, mL
 m = Sample mass, g
 m_{istd} = Mass of internal standard, g

METHOD OF ASSAY

Proceed as directed under Colouring Matters, Total Content by Spectrophotometry (procedure 2) in Volume 4, using the following conditions:

- W = amount to obtain adequate absorbance, g
 V1 = V2 = V3 = 100 mL
 v1 = v2 = 5 mL
 Solvent: cyclohexane
 $A_{1cm}^{1\%} = 2500$
 lambda max = 448 - 457 nm

FAST GREEN FCF

Prepared at the 84th JECFA and published in JECFA Monograph 20 (2017) superseding specifications prepared at the 30th JECFA (1986), revised at the 59th JECFA (2002), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005) and corrected at the 69th JECFA (2008). An ADI of 0-25 mg/kg bw was established at the 84th JECFA (2017).

SYNONYMS

INS No. 143, CI Food Green 3, CI (1975) No. 42053, FD&C Green No. 3

DEFINITION

Consists essentially of disodium 3-[*N*-ethyl-*N*-[4-[[4-[*N*-ethyl-*N*-(3-sulfobenzyl)amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]ammoniomethyl]benzenesulfonate and its isomers together with subsidiary colouring matters, as well as sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by condensing 2-formyl-5-hydroxybenzenesulfonic acid with a mixture of 3-[[*N*-ethyl-*N*-phenylamino)methyl]benzenesulfonic acid and its 2- and 4- isomers to form the leuco base precursor. Oxidation of the leuco base precursor with either chromium or manganese containing compounds produces the dye, which is purified and isolated as the disodium salt.

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-[*N*-ethyl-*N*-[4-[[4-[*N*-ethyl-*N*-(3-sulfobenzyl)amino]-phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-ammoniomethyl]benzenesulfonate;

N-ethyl-*N*-[4[[4-ethyl[(3-sulfophenyl)-methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide; inner salt, disodium salt

Disodium;2-[[4-[ethyl-[(3-sulfonatophenyl)methyl]amino]-phenyl]-4-[ethyl-[(3-sulfonatophenyl)methyl]azaniumylidene]-cyclohexa-2,5-dien-1-ylidene]methyl]-5-hydroxybenzene-sulfonate

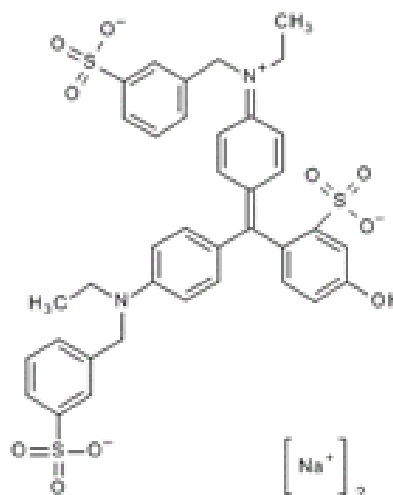
C.A.S. number

2353-45-9

Chemical formula

$C_{37}H_{34}N_2Na_2O_{10}S_3$

Structural formula



Formula weight	808.86
Assay	Not less than 85% total colouring matters
DESCRIPTION	Blue-green or red-brown powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Very soluble in water; slightly soluble in ethanol
<u>Spectrophotometry</u> (Vol. 4)	Maximum wavelength approximately 624 nm Determine the UV-visible absorption spectrum of the sample solution dissolved in water.
PURITY	
<u>Loss on drying, chloride and sulfate as sodium salts</u> (Vol. 4)	Not more than 15% as total amount Determine chloride as sodium chloride, sulfate as sodium sulfate, and water content (loss on drying at 135°) as described in Volume 4 (under "Specific Methods, Food Colours").
<u>Water insoluble matter</u> (Vol. 4)	Not more than 0.2%
<u>Subsidiary colouring matters</u>	Not more than 6% See description under TESTS
<u>Organic compounds other than colouring</u>	Not more than 0.5%, sum of 2-, 3-, and 4-formylbenzenesulfonic acids, sodium salts

<u>matters</u>	Not more than 0.3%, sum of 3- and 4-[<i>N</i> -ethyl- <i>N</i> -(4-sulfophenyl)amino]methylbenzenesulfonic acids, disodium salts
	Not more than 0.5% of 2-formyl-5-hydroxybenzenesulfonic acid, sodium salt
	See description under TESTS
<u>Leuco base</u> (Vol. 4)	Not more than 5.0% Weigh accurately 130±5 mg sample and proceed as directed under <i>Leuco Base in Sulfonated Triarylmethane Colours</i> (Vol. 4) Absorptivity (a) = 156 L/(g·cm) at 624 nm Ratio = 0.971
<u>Unulfonated primary aromatic amines</u> (Vol. 4)	Not more than 0.01% calculated as aniline
<u>Ether extractable matter</u> (Vol. 4)	Not more than 0.2%
<u>Lead</u> (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").
<u>Chromium</u> (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").
<u>Manganese</u> (Vol. 4)	Not more than 100 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").

TESTS

PURITY TESTS

<u>Subsidiary colouring matters</u> (Vol. 4)	Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions: <ul style="list-style-type: none"> - Column: C18 (150 mm x 2.1 mm i.d., 5 µm particle size) - Eluent A: 0.05 M ammonium acetate in water - Eluent B: 0.05 M ammonium acetate in methanol - Injection volume: 2 µl - Column temperature: 25° - Detector: UV-visible/PDA at 624 nm - Flow rate: 0.25 mL/min
----------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
8	63	37
16	55	45
33.5	49	51
48.5	0	100
48.6	100	0
60	100	0

Standards:

- Subsidiary colouring matters – synthesized materials
- Fast Green FCF (C.A.S. No. 2353-45-9) – TCI, Cat. No. F0146 or equivalent (use if subsidiary colouring matter standards are not available)

Sample preparation

Weigh accurately 500±5 mg sample and dissolve in 100 mL of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component.

Calculations

Construct the relevant standard curves. Integrate all peaks of the chromatograph obtained at 624 nm. If Fast Green FCF is used as the standard, calculate the ratio of the sum of all peaks not corresponding to Fast Green FCF to the sum of all peaks.

Organic compounds other than colouring matters (Vol. 4)

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

Detector: UV-visible/PDA at 246 nm

Standards:

- 2-Formylbenzenesulfonic acid, sodium salt (C.A.S. No. 1008-72-6) – Sigma-Aldrich, Cat. No. 12050 or equivalent (use for quantitating the 2-, 3-, and 4- isomers)

- 3-[[N-ethyl-N-(4-sulfophenyl)amino]methyl]benzenesulfonic acid, calcium salt (C.A.S. No. 5363-53-1, acid form) – Wako, Cat. No. 031-23071 or equivalent (use for quantitating the 3- and 4- isomers)
- 2-formyl-5-hydroxybenzenesulfonic acid, sodium salt (C.A.S. Nos. 106086-27-5, acid form; 119557-97-0, sodium salt) – Wako, Cat. No. 191-17231 or equivalent

Sample preparation

Weigh accurately 500 ± 5 mg sample and dissolve in 100 mL of water.

Calculations

Construct the relevant standard curves. Calculate the sum of 2-, 3-, and 4-formylbenzenesulfonic acids as their sodium salts, the sum of 3- and 4-[[N-ethyl-N-(4-sulfophenyl)amino]methyl]benzenesulfonic acids as their disodium salts, and 2-formyl-5-hydroxybenzenesulfonic acid as its sodium salt.

METHOD OF ASSAY (Vol. 4)

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

Using 0.04 M aqueous ammonium acetate as the solvent:
absorptivity (a) = 156 L/(g·cm) and wavelength of maximum absorbance = 624 nm.

GUM GHATTI

Prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017), superseding specifications prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI of 'not specified' was allocated at the 84th JECFA (2017)

SYNONYMS

Indian gum, ghatti gum, gum ghatti; INS No. 419

DEFINITION

Gum ghatti is a dried translucent exudate collected as 'tears' from the bark of the *Anogeissus latifolia* tree (family Combretaceae). It is manufactured by dissolving the tears in water followed by filtration and sterilization. The solution may be either dried to a gummy lump form or spray dried to a powder. It consists of complex high molecular weight water-soluble polysaccharides (on the order of several hundred kDa), present as calcium (or occasionally magnesium) salts. The product also contains small quantities of moisture, proteins, and tannins. The polysaccharides have a 1→6 linked β-D-galactose backbone with side chains containing L-arabinose units. The hydrolysis of the polysaccharide yields L-arabinose (~40%), D-galactose (~25%), D-glucuronic acid (~20%) and D-mannose (~7%), and small amounts of L-rhamnose (~1%) and D-xylose (~1%).

C.A.S. number

9000-28-6

DESCRIPTION

The gum ghatti product is a dried gummy lump or a spray dried powder. The unground product of gum ghatti occurs as amorphous tears of various sizes or in broken irregular pieces; light to dark brown. It is grey to reddish-grey in the powdered form. It has little or no odour.

FUNCTIONAL USES

Thickener, stabilizer, emulsifier and carrier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

When 1 g is dispersed in 5 mL of water it forms a viscous, adhesive mucilage; insoluble in ethanol

Gum constituents

L-Arabinose, D-galactose, D-mannose, L-rhamnose and D-xylose, and D-glucuronic acid should be present.
See description under TESTS

Optical rotation (Vol. 4)

A 1 in 50 aqueous solution of the sample filtered through diatomaceous earth is levorotatory.

Precipitate formation

To 5 mL of 1 in 100 solution of the sample (filter through

(Vol. 4) diatomaceous earth if necessary), add 0.2 mL of dilute lead subacetate TS. A slight precipitate may occur, and an opaque flocculent precipitate is formed upon further addition of 0.5 mL of ammonia TS.

PURITY

Loss on drying (Vol. 4) Not more than 14% (105°, 5 h)

Total ash (Vol. 4) Not more than 6%

Acid insoluble ash (Vol. 4) Not more than 1%

Lead (Vol. 4) Not more than 2 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 methods described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria (Vol.4) *Salmonella*: Negative in 25 g
E. coli: Negative in 1 g

TESTS

IDENTIFICATION TESTS

Gum constituents Detection of sugars (L-arabinose, D-galactose, D-mannose, L-rhamnose and D-xylose) and L-glucuronic acid by HPLC (Vol. 4)

Standards and reagents:

- L-Arabinose, D-galactose, D-mannose, L-rhamnose, D-xylose and L-glucuronic acid standards: >99% pure
- Trifluoroacetic acid, AR grade
- Sodium hydroxide: AR grade
- Deionized water: HPLC grade

Preparation of standard solution:

Dissolve and dilute to appropriate concentration using deionized water.

Preparation of sample solution:

Weigh about 50 mg of sample into a screw cap test tube with a PTFE liner. Add 4 mL deionized water and 0.5 mL trifluoroacetic acid. Close the screw-cap carefully and shake well. Place the test tube in an oven at 120° for 1 hour. Transfer the hydrolyzed sample to a 100 mL volumetric flask and make up to volume with deionized water. Dilute this solution 20 times and inject to HPLC.

Procedure:

- HPLC fitted with an anion-exchange column with pulsed amperometric detector, Dionex DX-50 or equiv.
- Column: Carbo-pack PA1, (250 mm x 4 mm, 5 µm, Dionex

- Co.) or equiv.
- Column temperature: 30°
- Mobile phase: 5 mM NaOH (A) and 1.0 M NaOH (B); use 100% A from 0 - 30 min and 100% B from 30 - 50 min (for wash) and return to 100% A until 70 min (system stabilization)
- Injection volume: 5 µl
- Flow rate: 1.0 mL/min

Inject individual standard solutions and record retention times of each component.

Inject prepared sample solution, dilute sample solution, if required and reinject. Identify the sugars and L-glucuronic acid from the retention times of the standards.

JAGUA (GENIPIN-GLYCINE) BLUE (TENTATIVE)

New specifications prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017). No ADI was established at the 84th JECFA (2017)

Information Required on:

- *Characterization of the low molecular weight components of the blue polymer*
- *A validated method for the determination of dimers*
- *Data for levels of dimers from five batches of the commercial product*

SYNONYMS

Jagua blue

DEFINITION

Jagua (Genipin-Glycine) Blue consists mainly of a blue polymer and blue dimers as minor subsidiary colours; it is produced by the reaction between genipin (methyl (1R,4aS,7aS)-1-hydroxy-7-(hydroxymethyl)-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-4-carboxylate) and glycine resulting in the combination of alternating dimeric moieties linked by a methylene bridge.

Jagua (Genipin-Glycine) Blue is produced by a two-step process. In the first step the unripe fruit of *Genipa americana* is peeled, ground to pulp, pressed for the juice, and extracted with water. The extracted juice, is filtered, and checked for genipin content. In the next step the jagua extract is treated with a stoichiometric amount of glycine and heated at 70° for two hours. The resulting liquid containing the Jagua (Genipin-Glycine) Blue is centrifuged and concentrated. A liquid product is obtained by concentrating the Jagua (Genipin-Glycine) Blue up to 20-50° Brix and formulating with glycerine or other food grade additives. Alternatively, a powder product is obtained after concentrating the Jagua (Genipin-Glycine) Blue 20° Brix and mixing with a food-grade carrier such as maltodextrin or modified food starches, spray drying, or using other drying technologies and sieving.

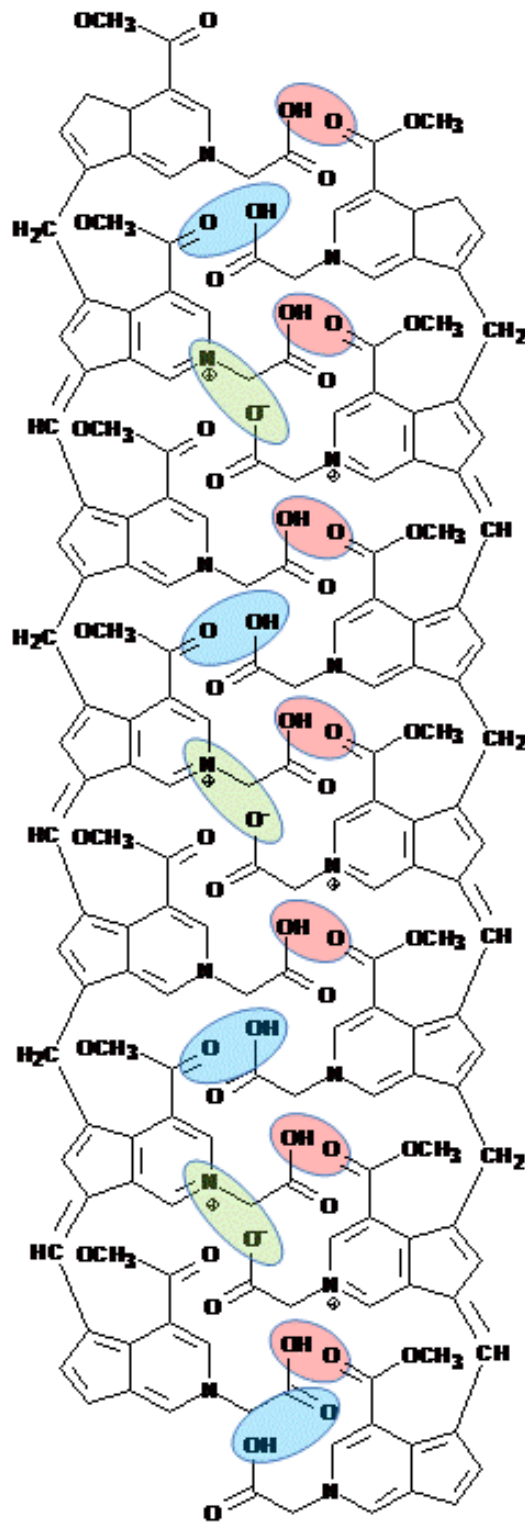
C.A.S. number

1314879-21-4
(Blue Polymer)

Chemical formula

$(C_{27}H_{25}O_8N_2)_n$
(n is typically 10-12)

Structural formula



Molecular weight

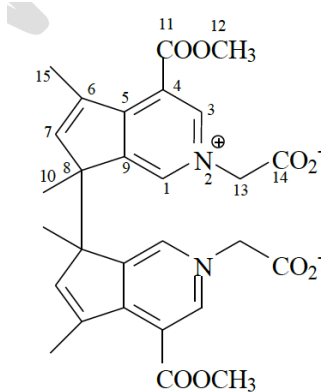
Approximately 6000 Da (number average molecular weight, approximately a lognormal distribution between 4500 and 9500 Da).

Assay Not less than 20% and not more than 40% as the polymer by HPLC
 Not less than 95% of % Total Colouring Matters
 See Methods of Assay

C.A.S. Number 1313734-13-2 (Dimer 1)

Chemical formula $C_{28}H_{28} N_2O_8$ (Dimer1)

Structural formula



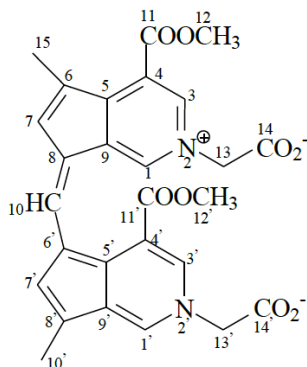
Dimer 1

Molecular weight 522 (Dimer1)

C.A.S. Number 104359-67-3 (Dimer 2)

Chemical formula $C_{27}H_{25} N_2O_8$ (Dimer 2)

Structural formula



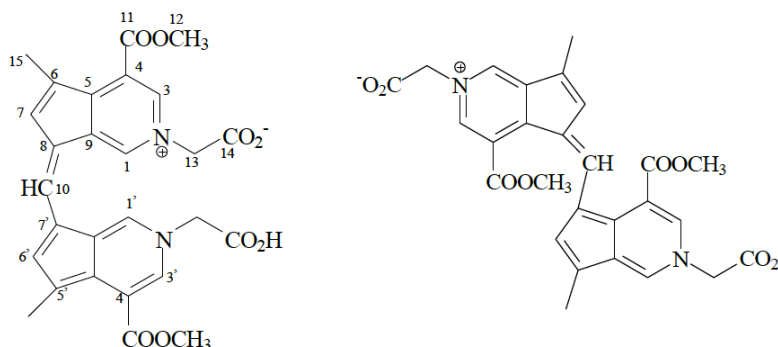
Dimer 2

Molecular weight 505 (Dimer 2)

C.A.S. Number 1313734-14-3 (Dimer 3)

Chemical Formula $C_{27}H_{24}N_2O_8$ (Dimer 3)

Structural Formula



Dimer 3

Molecular weight

505 (Dimer 3)

Assay

*Information required for Dimer 1, Dimer 2 and Dimer 3***DESCRIPTION**

Blue to black, liquid or in powder; odourless

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Freely soluble in water. Practically insoluble in hexane and ethanol.

Spectrophotometry (Vol.4)

The UV-Visible absorption spectrum of a sample dissolved in water shows absorption maximum between 590-594 nm.

HPLC

The retention time of the blue polymer is approximately at 10 min ($\lambda_{\text{detector}}=590$ nm); it is the main peak in the chromatogram of Jagua (Genipin-Glycine) Blue.
See Method of Assay.

Infrared Spectrum

Infrared spectrum of the sample obtained by using potassium bromide disk corresponds to the reference spectrum

PURITY

Loss on drying (Vol 4)

Not more than 6% (at 105°, to constant weight).

Water insoluble matter (Vol. 4)

Not more than 0.2%

<u>Ether-extractable Matter</u> (Vol. 4)	Not more than 0.2%
<u>Genipin</u>	Not more than 0.3% See description under TESTS
<u>Arsenic</u> (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
<u>Lead</u> (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
<u>Microbiological Criteria</u> (Vol 4)	Aerobic Plate Count: Not more than 1000 CFU/g Total Coliforms : Not more than 10 CFU/g <i>E. coli</i> : Absent in 25 g of sample Coagulase positive <i>S. aureus</i> : Absent in 1 g of sample Yeasts and moulds: Not more than 10 CFU/g

TESTS

PURITY TESTS

<u>Genipin</u>	HPLC Analysis of Genipin in Jagua (Genipin-Glycine) Blue <ul style="list-style-type: none"> - Apparatus: HPLC-UV-Vis: - Column: Phenomenex Luna PFP (PentaFluoroPhenyl, 100Å, 150 x 4.6 mm, 5µm), with guard column, or equivalent. - Flow Rate: 1 mL/min - Injection volume: 10 µL - Eluents: A) 0.1% acetic acid in water; B) Methanol, HPLC grade - Detector: UV at 240 nm for peak identification and quantitation - Temperatures: <ul style="list-style-type: none"> o T_{Column}: 30° o T_{Detector}: 30° - Retention time: approximately 22 minutes - Standard: Genipin (>98%, HPLC grade)
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Gradient:

Time (min)	% Solvent A	% Solvent B
0	95	5
15	65	35
22	65	35
27	0	100
29	0	100
31	95	5
41	95	5

Sample Preparation:

Dissolve 10 mg of Jagua (Genipin-Glycine) Blue in ethanol:water (1:1) in a 10 mL volumetric flask. Transfer 6 mL of the above solution to a 10 mL volumetric flask and fill to the mark with a methanol:water (1:1) solution. Filter with 0.45 µm nylon filter and inject onto the HPLC column.

Quantitation:

Construct the linear standard curve for quantitation of genipin using the equation:

$$\text{Peak Area} = \text{Slope} \times [\text{Genipin}], \mu\text{g/mL} + \text{Intercept}$$

Use the following for quantitation

$$\% \text{Genipin} = \frac{(\text{Area at } 240 \text{ nm} - \text{Intercept})}{\text{Slope}} \times \frac{10}{(6 \times \text{mg of sample})}$$

METHOD OF ASSAY

The percentage of the blue polymer in Jagua (Genipin-Glycine) Blue is calculated by HPLC (Vol. 4).

The contribution to colour from the blue polymer is calculated as %Total Colouring Matters by spectrophotometry (Vol. 4).

Preparation of In-House Reference Standard

Equipment: Glass column; resin Sephadex LH-20; RP-C18 Column.

Solvents: water, methanol.

STEP 1: Wash about 5 g of Jagua (Genipin-Glycine) Blue powder, at least twice, with 200 mL ethyl acetate and discard the supernatant.

STEP 2: Extract the residue from Step 1 with 500 mL methanol, at least five times. Separate the remaining solid material and save for Step 4. Reduce the volume of the supernatant under vacuum. Load this on to a Sephadex LH-20 Column.

STEP 3: Elute with methanol:H₂O (50:50) to obtain two coloured fractions:
 Fraction 1 (about 600 mg). Reduce the volume using vacuum and save this fraction for Step 5.
 Fraction 2 (about 200 mg). Load this fraction on to a RP-C18 Column. Elute with a MeOH gradient (50% up to 100%) in order to obtain 3 coloured fractions corresponding to dimers 1, 2 and 3.

STEP 4: Load the residue from Step 2 (about 1.2 g) on to the Sephadex LH-20 column. Elute with MeOH:H₂O (9:1).

STEP 5: Combine the first blue band of Step 4 with Fraction 1 of Step 3. Run the combined fraction on a RP C-18 column chromatography separation, and elute with MeOH:H₂O (2:1). Combine the three blue bands obtained. Lyophilize, and label the residue as In-House Reference Standard.

Preparation of the Standard Solution

Accurately weigh 10 mg of the In-house Reference Standard. Transfer to a 20 mL volumetric flask and dissolve with deionized water. Prepare a minimum of five point standard curve by serially diluting the Reference Standard Stock with deionized water in duplicate.

Qualitative and quantitative determination of blue polymer in Jagua (Genipin-Glycine) Blue

Apparatus:

- HPLC- Photodiode Array (PDA)/UV-Vis
- Column: Phenomenex Luna PFP (PentaFluoroPhenyl, 100 Å, 150 x 4.6 mm, 5 µm) with guard column, or equivalent.
- Flow Rate: 1 mL/min
- Injection volume: 10 µl
- Eluents: A) Water; B) Methanol, HPLC grade
- Detector: PDA/UV-Vis (wavelengths: 230-800 nm; 590 nm for peak identification and quantitation)
- Temperatures:
 - o T_{Column}: 40°
 - o T_{Detector}: 40°
- Sample: Room Temperature
- Retention Time of interest: 10.3 min

Gradient:

Time (min)	% Solvent A	% Solvent B
0	80	20
5	80	20
6	0	100
10	0	100
11	80	20
20	80	20

Sample Preparation:

Prepare three independent samples dissolving 10 mg each of Jagua Blue in deionized water (10 mL volumetric flask). Filter before injection onto column.

Quantitation:

Refer to the standard curve described in the "Method of Assay" to calculate the (%) concentration of the polymer in Jagua blue using the following formula:

$$\% \text{ Polymer} = \frac{\text{Area at } 590 \text{ nm} \pm \text{Intercept}}{\text{Slope}} \times \frac{100}{1000} \times \frac{10}{w}$$

Where Intercept and Slope are the values obtained from the standard curve and w is the weight (mg) of the sample.

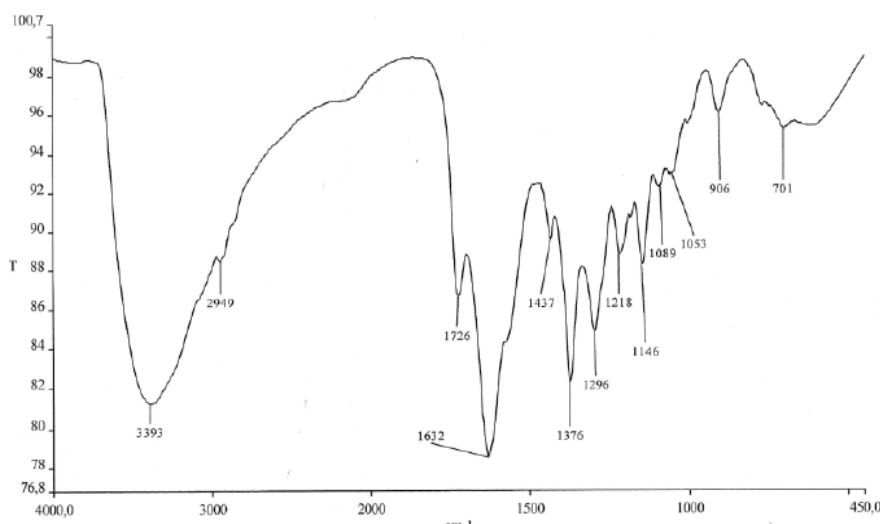
% Total Colouring Matters

Determine % of Total Colouring Matters by Spectrophotometry using water as solvent, as described in Volume 4 of Combined Compendium of Food Additive Specifications (Food Colours, Procedure 1). $a=14.911 \text{ L/(g}\cdot\text{cm)}$ and $E^{1\%}_{1\text{cm}}=149.1$ of the polymer at the wavelength of maximum absorbance (592 nm), respectively.

$$\% \text{ Total Colouring Matters} = 100 \times \frac{\text{Abs} \times 1L}{a \times 1\text{cm}} \times \frac{F}{w}$$

Where:

- Abs is the absorbance of the sample solution at 592 nm
- a is the absorptivity of the standard solution (L/g*cm)
- F is the dilution factor
- W grams of the sample.



IR Spectrum of the blue polymer in Jagua (Genipin-Glycine) Blue shows characteristic bands at: 3393, 2949, 1726, 1630 and 1540 cm^{-1} .

METATARTARIC ACID (TENTATIVE)

New tentative specifications prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017). The 84th JECFA concluded that metatartaric acid (when used in winemaking) is included in the group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium, potassium–sodium salts, expressed as L(+)-tartaric acid.

Information required on:

- *Characterization of the product (optical rotation, content of free tartaric acid, degree of esterification and molecular weight distribution)*
- *IR spectrum (in a suitable medium)*
- *Analysis results including above parameters from a minimum of 5 batches of products currently available in commerce along with QC data.*

The Committee requests that this information be submitted by December 2018.

SYNONYMS

INS No. 353

DEFINITION

Metatartaric acid is a polydisperse polymer of tartaric acid with a degree of esterification above 32%. It is manufactured by heating L-tartaric acid from natural sources at temperatures of 150-170° under atmospheric or under a reduced pressure. The product contains di-tartaric monoester and diester, other polyester acids of variable chain length, as well as non-esterified tartaric acid.

Chemical name

Metatartaric acid

C.A.S. number

56959-20-7/ 39469-81-3

Chemical formula

$(C_4H_4O_5)_n$

Assay

Not less than 105% as total tartaric acid

DESCRIPTION

Crystalline or powder form with an off-white colour. Very deliquescent with a faint odour of caramel

FUNCTIONAL USES

Stabilizer (prevents growth and precipitation of potassium bitartrate and calcium tartrate crystals in wine)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water and soluble in ethanol

pH (Vol. 4) pH of 1% solution = 1.4 - 2.1

Infra-red spectrum (Vol.4) Information required

Test for tartrate (Vol. 4) Passes test

PURITY

Loss on drying Not more than 5% at 105⁰, 2h

Free tartaric acid Not more than XX % (*Information required*)
See description under METHOD OF ASSAY

Degree of esterification Not less than 32% (See description under METHOD OF ASSAY)

Arsenic (Vol. 4) Not more than 3 mg/kg
Determine using a method appropriate to the specified level. Use Method II to prepare sample solution. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Lead (Vol. 4) Not more than 2 mg/kg
Determine using 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").

METHOD OF ASSAY Treating metatartaric acid with sodium hydroxide will cause de-esterification of metatartaric acid resulting in tartaric acid. This will allow calculation of the degree of esterification. Addition of a known excess of sodium hydroxide solution followed by back titration with standard sulfuric acid to ~ pH=7 (bromothymol blue indicator) will allow calculation of the total free and esterified acid present in the sample.

Reagents:

- Standard sodium hydroxide solution, 1 M
- Standard sulfuric acid solution, 0.5 M
- Bromothymol blue TS

Procedure:

Accurately weigh about 20 g of metatartaric acid (W), dissolve in deionized water and make up to volume in a 1 L volumetric flask, and mix well. Pipette 50 mL of this solution into a Erlenmeyer flask

Add about 10 drops of bromothymol blue TS and mix well.

Titrate with 1 M sodium hydroxide solution until the indicator turns bluish-green (pH=7). Record the titer value, mL (n).

Pipette 20 mL of 1 M sodium hydroxide into the Erlenmeyer flask, stopper and allow to stand for 2 hours at ambient temperature.

Titrate with 0.5 M sulfuric acid until the indicator turns bluish-green (pH=7). Record the titer value, mL (n').

Calculation:

Free tartaric acid: $F (\%w/w) = 150.09 \times n/W$

Esterified tartaric acid: $P (\%w/w) = 150.09 (20-n')/W$

Where:

- W is the weight of sample
- Total tartaric acid, $\%w/w = F+P$
- Degree of Esterification (%) = $100 (20-n')/[n+(20-n')]$

Appendix

Infra-red spectrum of pure metatartaric acid (*Information required*)

MICROCRYSTALLINE CELLULOSE

Prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017), superseding specifications prepared at the 55th JECFA (2000) and published in Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1, Vol. 2 (2006). An ADI "Not specified" was established at the 49th JECFA (1998).

SYNONYMS

Cellulose gel; INS No. 460(i)

DEFINITION

Purified, partially depolymerized cellulose prepared by treating alpha- cellulose, obtained as a pulp from fibrous plant material, with mineral acids. The degree of polymerization is typically less than 400. Not more than 10% of the particles have a diameter below 5 µm.

Chemical names

Cellulose

C.A.S. number

9004-34-6

Chemical formula

 $(C_6H_{10}O_5)_n$

Assay

Not less than 97% of carbohydrate calculated as cellulose on the dry basis.

DESCRIPTION

Fine, white or almost white, odourless, free flowing crystalline powder.

FUNCTIONAL USES

Emulsifier, stabilizer, anticaking agent, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water and ethanol. Practically insoluble or insoluble in sodium hydroxide solution (50 g/L)

Infrared absorption

The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to the infrared spectrum below.

Suspension formation

Mix 30 g of the sample with 270 mL of water in a high-speed (18,000 rpm) blender for 5 min. Transfer 100 mL of the mixture to a 100-mL graduated cylinder, and allow to stand for 3 h. A white, opaque, bubble- free dispersion that forms a supernatant, is obtained.

PURITY

Loss on drying
(Vol. 4)

Not more than 7.0% (105°, 3 h)

pH (Vol. 4)

5.0 - 7.5

Shake 5 g of the sample with 40 mL of water for 20 min and centrifuge. Determine the pH of the supernatant.

Sulfated ash (Vol. 4)

Not more than 0.05%

Test 10 g of the sample (Method I)

Water soluble
Substances

Not more than 0.24%.

Shake 5 g of the sample with approximately 80 mL of water for 10 min, filter through Whatman No. 42 or equivalent filter paper into a tared beaker, wash residue with 20 mL of water and evaporate to dryness on a steam bath. Dry at 105° for 1 h, cool, weigh and calculate as percentage.

Starch

Not detectable

To 20 mL of the dispersion obtained in the identification test for starch, add a few drops of iodine TS, and mix. No purplish to blue or blue colour should be obtained.

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 principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

METHOD OF ASSAY

Transfer about 125 mg of the sample, accurately weighed, to a 300 mL Erlenmeyer flask, using about 25 mL of water. Add 50.0 mL of 0.5N potassium dichromate and mix. Carefully add 100 mL of sulfuric acid and heat to boiling. Remove from heat, allow to stand at room temperature for 15 min and cool in a water bath. Transfer the contents into a 250 mL volumetric flask, rinse flask with distilled water, add rinsings to the volumetric flask and dilute with water almost to volume. Allow the volumetric flask to reach room temperature (25°); then make up to volume with water and mix. Titrate a 50.0 mL aliquot with 0.1 N ferrous ammonium sulfate using 2 or 3 drops of ortho-phenanthroline TS as the indicator and record the volume required as S in mL. Perform a blank determination and record the volume of 0.1 N ferrous ammonium sulfate required as B in mL.

Calculate the percentage of cellulose in the sample using the formula:

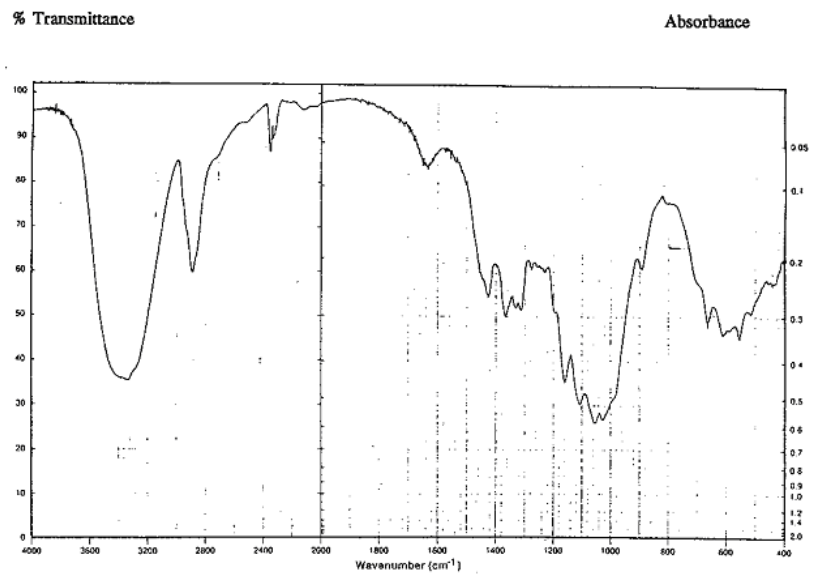
$$(B - S) \times \frac{338}{W}$$

Where:

- W is the weight of sample taken, in mg, corrected for loss on drying.

Appendix

Infrared spectrum of microcrystalline cellulose



SILICON DIOXIDE, AMORPHOUS

Prepared at the 84th JECFA and published in FAO JECFA Monographs 20 (2017), superseding tentative specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI 'not specified' for silicon dioxide and certain silicates was established at the 29th JECFA (1985).

SYNONYMS

Silica; INS No. 551; Synthetic amorphous silica (SAS); Silicon dioxide

DEFINITION

Silicon dioxide is an amorphous substance, which is produced synthetically by either a thermal process, yielding pyrogenic (fumed) silica, or by a wet process, yielding hydrated silica, precipitated silica and silica gel. Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.

Chemical names

Silicon dioxide, chemically prepared

C.A.S. number

7631-86-9

112945-52-5 (pyrogenic silica)

112696-00-8 (hydrated silica)

Chemical formula

$(\text{SiO}_2)_x$

Formula weight

60.08 (SiO_2)

Assay

Pyrogenic (fumed) silica:
Not less than 99% of SiO_2 on the ignited basis

Hydrated silica (precipitated silica and silica gel):
Not less than 94% of SiO_2 on the ignited basis

DESCRIPTION

Pyrogenic (fumed) silica:
A pyrogenic silicon dioxide occurring as a fine, white amorphous powder or granules

Hydrated silica (precipitated silica and silica gel):
A precipitated, hydrated silicon dioxide occurring as a fine, white amorphous powder or granules

FUNCTIONAL USES

Anticaking agent, carrier

CHARACTERISTICS

IDENTIFICATION

<u>Test for silicon</u>	Passes test See description under TESTS
<u>Solubility (Vol. 4)</u>	Insoluble in water and insoluble in ethanol
<u>pH (Vol. 4)</u>	Pyrogenic (fumed) silica: 3.0 – 5.0 (5% slurry, 20°) Hydrated silica (precipitated silica and silica gel): 4.0 - 9.0 (5% slurry, 20°)

PURITY

<u>Loss on drying (Vol. 4)</u>	Pyrogenic (fumed) silica: Not more than 2.5% (105°, 2 h) Hydrated silica (precipitated silica and silica gel): Not more than 8% (105°, 2 h)
<u>Loss on ignition (Vol. 4)</u>	Pyrogenic (fumed) silica: Not more than 2.5% (1000°, 1h on dried sample) Hydrated silica (precipitated silica and silica gel): Not more than 8.5% (1000°, 1 h on dried sample)
<u>Impurities soluble in 0.5M hydrochloric acid</u>	Lead: Not more than 3 mg/kg Arsenic: Not more than 1 mg/kg Determine using a method appropriate to the specified level. See description under TESTS for sample preparation

TESTS

IDENTIFICATION TESTS

<u>Test for silicon</u>	Prepare the test solution as shown under method of assay. Analyse silicon in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Si (251.611 nm).
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PURITY TESTS

<u>Impurities soluble in 0.5 M hydrochloric acid</u>	Extract a known quantity of finely ground sample in a closed digestion system with 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid and dilute to a known volume with 0.5 M hydrochloric acid.
------------------------------------------------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

METHOD OF ASSAY

Accurately weigh an appropriate quantity of the sample, (depending on the moisture content) equivalent to about 0.5 g of dried sample, in a platinum or nickel crucible, add 5 g potassium

hydroxide and 2 g boric acid, mix and melt completely using a torch burner and allow to stand at room temperature.

Place the reaction product along with crucible into 150 mL hot deionized water in a 250-mL PTFE beaker and dissolve residue by agitation. Wash the crucible with hot deionized water and remove it. Add 50 mL hydrochloric acid and transfer the contents into a 250-mL polypropylene volumetric flask. Wash the beaker three times with hot deionized water. Transfer the washings to the volumetric flask and make up to volume (Solution A).

Prepare the test solution by diluting Solution A with 2% hydrochloric acid, to get the readings within the standard curve range.

Analyse silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical line for Si (251.611 nm) and construct standard curve using standard solutions 0.1 – 5.0 µg/mL.

Read the concentration of Si in test solution (as µg/mL) and calculate the silicon dioxide content of the sample using the formula:

$$\text{SiO}_2 (\%) = (2.139 \times C \times 250 \times \text{DF}) / (W \times 106) \times 100$$

Where:

- C is concentration of Si in the test solution, µg/mL;
- DF is dilution factor (dilution of solution A to get test solution);
- W is weight of sample on the ignited basis, g

SODIUM ALUMINIUM SILICATE

Prepared at the 84th JECFA and published in FAO JECFA Monograph 20 (2017), superseding tentative specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI 'not specified' for silicon dioxide and certain silicates was established at the 29th JECFA (1985). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.

SYNONYMS	Sodium silicoaluminate; sodium aluminosilicate; aluminium sodium silicate; silicic acid, aluminium sodium salt; INS No. 554
DEFINITION	Sodium aluminium silicate is a series of amorphous hydrated sodium aluminium silicates with varying proportions of Na ₂ O, Al ₂ O ₃ and SiO ₂ . It is manufactured by reacting aluminium sulphate and sodium silicate followed by precipitation.
Chemical names	Aluminium sodium silicate
C.A.S. number	1344-00-9
Chemical formula	xSiO ₂ · yAl ₂ O ₃ · zNa ₂ O
Assay	Silicon dioxide (SiO ₂): Not less than 66% and not more than 88%. Aluminium oxide (Al ₂ O ₃): Not less than 5% and not more than 15%. Sodium oxide (Na ₂ O): Not less than 5% and not more than 8.5%. All values expressed on the dried basis.
DESCRIPTION	Odourless, fine, white amorphous powder, or as beads
FUNCTIONAL USES	Anticaking agent
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Insoluble in water.
<u>Test for sodium</u>	Passes test See description under TESTS
<u>Test for aluminium</u>	Passes test See description under TESTS

Test for silicon Passes test
See description under TESTS

PURITY

pH (Vol. 4) 6.5 – 11.5 (5% slurry)

Loss on drying (Vol. 4) Not more than 8.0% (105°, 2 h).

Loss on ignition (Vol. 4) Not less than 5.0% and not more than 11.0% on the dried basis (1000°, constant weight).

Impurities soluble in 0.5 M hydrochloric acid
Lead: Not more than 5 mg/kg
Arsenic: Not more than 3 mg/kg
Mercury: Not more than 1 mg/kg

See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for aluminium, sodium and silicon
Prepare the test solution as shown under method of assay. Analyse aluminium, sodium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use analytical lines for Al (396.15 nm), Na (589.52 nm) and Si (251.611 nm).

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid
Extract a known quantity of finely ground sample using closed system digestion (to prevent loss of arsenic and mercury) with 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with 0.5 M hydrochloric acid. Combine the filtrate and wash solution and make up to a known volume with 0.5 M hydrochloric acid.
Determine using a method appropriate to the specified level

METHOD OF ASSAY

Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, mix and melt completely using a torch burner and allow to stand at room temperature.

Place the reaction product along with crucible into 150 mL hot deionized water in a 250-mL PTFE beaker and dissolve residue by agitation. Wash the crucible with hot deionized water and remove it. Add 50 mL hydrochloric acid and transfer the contents into a 250-mL polypropylene volumetric flask. Wash the beaker

three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume.

Dilute with 2% hydrochloric acid and prepare the test solution. Analyse aluminium, sodium, and silica in the test solution by ICP-AES technique (Vol. 4).

Set instrument parameters as specified by the instrument manufacturer. Use analytical lines for Al (396.152 nm), Na (589.52 nm) and Si (251.611 nm).

Construct standard curve using standard solutions 0.2 – 5.0 µg/mL each. Read the concentration of Al, Na and Si, in sample solution (as µg/mL). Conduct as a blank determination following the above procedure.

Calculate the content of aluminium oxide, sodium oxide and silicon dioxide in the sample using the formulas:

$$Al_2O_3 (\%) = \frac{1.889 \times (C - B) \times 250 \times DF}{W \times 10^6} \times 100$$

$$Na_2O (\%) = \frac{1.348 \times (C - B) \times 250 \times DF}{W \times 10^6} \times 100$$

$$SiO_2 (\%) = \frac{2.139 \times (C - B) \times 250 \times DF}{W \times 10^6} \times 100$$

Where:

- C (µg/mL) is concentration of Al or Na or Si in the test solution,
- B (µg/mL) is concentration of Al or Na or Si in the blank solution,
- W is weight of sample on the ignited basis,
- DF is dilution factor

STEVIOL GLYCOSIDES FROM *STEVIA REBAUDIANA* BERTONI

Prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017), superseding tentative specifications prepared at the 82nd JECFA (2016) and published in FAO JECFA Monographs 19 (2016). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS

INS No. 960

DEFINITION

Steviol glycosides consist of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose, arabinose, galactose and deoxyglucose) in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni. The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Chemical name

See Appendix 1

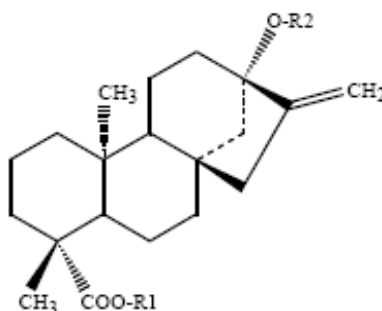
C.A.S. number

See Appendix 1

Chemical formula

See Appendix 1

Structural formula



Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.

Glc, Rha, Fru, deoxyGlc, Gal, Ara and Xyl represent, respectively, glucose, rhamnose, fructose, deoxyglucose, xylose, galactose, arabinose and xylose sugar moieties.

Assay

Not less than 95% of total of steviol glycosides, on the dried basis, determined as the sum of all compounds containing a

steviol backbone conjugated to any number, combination or orientation of saccharides (glucose, rhamnose, fructose, deoxyglucose xylose, galactose, arabinose and xylose) occurring in the leaves of *Stevia rebaudiana* Bertoni.

DESCRIPTION

White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES

Sweetener

CHARACTERISTICS**IDENTIFICATION**Solubility (Vol. 4)

Freely soluble in a mixture of ethanol and water (50:50)

HPLC chromatographic profile

The main peaks in a chromatogram obtained by analysing a sample following the procedure in METHOD OF ASSAY correspond to steviol glycosides

pH (Vol. 4)

Between 4.5 and 7.0 (1 in 100 solution)

PURITYTotal ash (Vol. 4)

Not more than 1%

Loss on drying (Vol. 4)

Not more than 6% (105°, 2 h)

Residual solvents (Vol. 4)

Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I, General Methods, Organic Components, Residual Solvents)

Arsenic (Vol. 4)

Not more than 1 mg/kg
Determine using a method appropriate to the specified level (Use Method II to prepare sample solution). 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").

Microbiological criteria (Vol. 4)

Total (aerobic) plate count: Not more than 1,000 CFU/g
Yeasts and moulds: Not more than 200 CFCU/g

E. coli: Negative in 1 g
Salmonella: Negative in 25 g

METHOD OF ASSAY

Determine the percentages of major steviol glycosides (those with analytical standards) using Method A (HPLC, Vol. 4). Confirm the presence of each minor steviol glycoside (compounds where analytical standards are not available) using Method B (HPLC-MS). Calculate the concentration of the minor compounds using respective molecular mass corrected UV peak area against the rebaudioside A UV standard curve. Calculate their sum and express the content on the dried basis.

Method A: Determination of Major Steviol Glycosides by HPLC:

Reagents:

- Acetonitrile: HPLC grade with transmittance more than 95% at 210 nm.
- Deionized water: HPLC grade
- Standards (Reference and Quality Control Standards): Stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, rebaudioside M, rebaudioside N, rebaudioside O, dulcoside A, rubusoside and steviolbioside. Chromadex, USA; Wako Pure Chemical Industries Ltd., Japan; Sigma-Aldrich; US Pharmacopeia or equivalent.

Note: Standards of other steviol glycosides, which may become commercially available in the future, may also be included. The analyst should consider that the inclusion of additional standards will lower the concentration of the mixed standards described below.

Preparation of Steviol Glycosides Standard Solutions:

Prepare individual stock standard solutions (1.5 mg/mL) in water:acetonitrile (7:3)

Prepare mixed standard solution (115 µg/mL) by mixing 1.0 mL each individual stock standard solutions.

Prepare Peak Identification Standard Solutions (0.1 mg/mL) from individual stock standard solutions in water:acetonitrile (7:3).

Prepare mixed working standard solutions in the range of 20 – 100 µg/mL by following appropriate dilution of mixed standard solution (b) with water:acetonitrile (7:3).

Prepare quality control and system suitability individual stock standard solutions (1.5 mg/mL) as well as mixed standard solution (115 µg/mL) using standards from a different batch /manufacturer (if available).

Prepare quality control mixed working standard solutions (40 and 80 µg/mL) and system suitability standard (52 µg/mL) by following appropriate dilutions of mixed standard solution

Preparation Sample Solution:

Accurately weigh 50 mg of sample and quantitatively transfer into a 50-mL volumetric flask. Add about 20 mL of water:acetonitrile (7:3), sonicate and shake well to dissolve the sample and make up to volume.

Procedure:

Use a HPLC consisting of a high precision binary pump and an auto sampler (capable of operating at 2 -8°); Diode-Array detector @ UV at 210 nm; and Mass Spectrometric Detector (Electrospray Negative Ionisation over a mass range from 50 to 1500 m/z using a unit mass resolution, For use in Method B below) connected in series. Agilent 1200 with Waters Quattro or equivalent:

- Column: Luna 5µ C18(2), 100A, (150 mm x 4.6 mm, 5µm, Phenomenex) or Capcell pak C₁₈ MG II (250 mm x 4.6 mm, 5µm, Shiseido Co. Ltd) or equiv.
- Column temperature: 50°
- Autosampler temperature: 2 – 8°
- Injection volume: 10 µl
- Mobile phase A: Deionised or LC-MS grade water (0.2 µm filtered)
- Mobile phase B: LC-MS grade Acetonitrile (0.2 µm filtered)

HPLC Gradient Time table:

Time (min)	% Solvent A	% Solvent B	Flow Rate (mL/min)
0.00	85.0	15.0	0.3
40.0	70.0	30.0	0.3
60.0	55.0	45.0	0.3
70.0	55.0	45.0	0.3
70.1	85.0	15.0	0.3
80.0	85.0	15.0	0.3

Inject peak identification standard solutions (c), identify peaks and calculate relative retention times (RRT) with respect to rebaudioside A (Typical RRT values are given in Appendix-3). See Appendix 2 for an example of a chromatogram obtained using the method.

Inject working mixed standard solutions (d) and construct standard curves for each steviol glycoside. Inject quality

control and system suitability standard solutions (f) to ensure a satisfactory working system.

Inject prepared samples. Dilute sample solution, if required, to bring the concentration of each analyte within the standard curve range. Make duplicate injections. Deduce concentration of each steviol glycoside from its corresponding standard curve and obtain average concentration in sample solution ($\mu\text{g/mL}$).

Calculation of major steviol glycosides content:

Calculate the concentration of each steviol glycoside in the sample solution using the following formula:

$$\text{Conc (\%w/w)} = c_{\text{sample}} \times \frac{100}{W_{\text{sample}}}$$

Where:

- C_{sample} is the average concentration ($\mu\text{g/mL}$) in the sample solution
- W_{sample} is the weight of sample (μg) in 1 mL of sample solution ($\sim 1000 \mu\text{g/mL}$)

Note: Above calculation will change if additional dilutions were done prior to LC injection. Analyst shall account such dilutions in the calculation.

Calculate the percentage of major steviol glycosides in the sample by summation of percentages of individual steviol glycosides in the sample (A).

Note: If the concentration of major steviol glycosides in the sample is <95%, then analyst should perform Method B.

Method B: Determination of Minor Steviol Glycosides by HPLC-MS:

HPLC-MS conditions may vary based on the manufacturer and model of the system used. Analyst should set the conditions following the manufacturer's instructions. Typical HPLC-MS Conditions for Waters Quattro Micro mass spectrometer are shown in the Annexure.

The mass spectrometer is connected to the HPLC-UV system used in method A. Analyse the mass spectral data of the minor peaks (major steviol glycoside peaks are identified from RRT in method A). Confirm the presence of each minor steviol glycoside from the observed molecular mass ion (Typical molecular mass ions of steviol glycosides are given in Appendix-3) and one or more of the following mass spectral diagnostic ions:

Mass spectral diagnostic ions observed during in-source fragmentation of steviol glycosides

[Fragment-H] - m/z	Identity
317	Steviol
427	Related Steviol glycoside #3
479	Steviol-GLC
625	Steviol-2GLC [M-16]
641	Steviol-2GLC
787	Steviol-3GLC deoxyglucose [M-16]
803	Steviol-3GLC
819	-
965	Steviol-4GLC

Note: The example chromatogram of minor steviol glycosides shown in Appendix 2 is obtained from the purified in-house standards.

After confirming the presence of a minor steviol glycoside, correct its mean peak area (obtained from the UV chromatogram) as described below.

Calculation of minor steviol glycosides content:

Calculate the molecular mass corrected peak area abundance for each minor steviol glycoside using the formula:

$$\text{Molecular mass corrected peak area} = \frac{M_x \times \text{MPA}}{M_{\text{RebA}}}$$

Where:

- M_x is the molecular mass of the minor steviol glycoside
- M_{RebA} is the molecular mass of Rebaudioside A (967 amu)
- MPA is the mean peak area

Deduce the concentration ($\mu\text{g/mL}$) of each minor steviol glycoside using from the UV standard curve of rebaudioside A. Calculate the concentration of each minor steviol glycoside in the sample solution using the following formula:

$$\text{Minor Steviol Glycoside Conc. (\%w/w)} = \frac{\text{Conc}_{\text{sample}} \times 100}{\text{Weight}_{\text{sample}}}$$

Where

- $\text{Conc}_{\text{sample}}$ is the assayed concentration ($\mu\text{g/mL}$) in the test sample
- $\text{Weight}_{\text{sample}}$ is the sample weight in 1 mL solution ($\mu\text{g/mL}$)

Note: Above calculation will change if additional dilutions were done prior to LC injection. Analyst shall account such dilutions in the calculation.

Calculate the percentage of minor steviol glycosides in the sample by summation of percentages of individual minor steviol glycosides in the sample (B).

Determine the total amount of steviol glycoside content using the following formula:

$$TSG = \frac{(A + B) \times 100}{(100 - M)}$$

Where:

- TSG is the Total steviol glycosides content (%w/w, on the dried basis)
- A is the percent major steviol glycosides
- B is the percent minor steviol glycosides
- M is the percent loss on drying

Annex Typical LCMS Conditions

Instrumentation	Waters Quattro Micro mass spectrometer
Ionization:	Electrospray negative polarity
Capillary voltage:	4.0 kV
Cone voltage:	35 V (low) and 60 V (high)
Extractor voltage:	5.0 V
RF lens voltage:	1.0 V
Source temperature:	90 °
Desolvation temperature:	350 °
Desolvation flow rate:	400 L/h
Collisional pressure:	Not applicable
Collisional voltage:	Not applicable
Collision gas:	Not applicable
Resolution:	1 amu
Data acquisition	Scanning from 50 to 1500 m/z using Mass Lynx

Appendix 1: Chemical Information of Some Steviol Glycosides

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Group 1: Steviol + Glucose (SvGn)							
Steviolmonoside	SVG1	H	Glcβ1-	13-[β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid	60129-60-4	C ₂₆ H ₄₀ O ₈	481
Steviolmonoside A	SVG1	Glcβ1-	H	13-[(hydroxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64977-89-5	C ₂₆ H ₄₀ O ₈	481
Rubusoside	SVG2	Glcβ1-	Glcβ1-	13-[β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64849-39-4	C ₃₂ H ₅₀ O ₁₃	643
Steviolbioside	SVG2	H	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid	41093-60-1	C ₃₂ H ₅₀ O ₁₃	643
Stevioside	SVG3	Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	57817-89-7	C ₃₈ H ₆₀ O ₁₈	805
Stevioside A Or Rebaudioside KA	SVG3	Glcβ(1-2)Glcβ1-	Glcβ1-	13-[(2-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid 4)-O-β-D-glucopyranosyl-deoxy-(1,2)-O-[β-(d-glucopyranosyl ester	127345-20-4	C ₃₈ H ₆₀ O ₁₈	805
Stevioside B	SVG3	Glcβ(1-3)Glcβ1-	Glcβ1-	13-[(2-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, O-β-D-glucopyranosyl-deoxy-(1,3)-O-[β-D-glucopyranosyl ester	-	C ₃₈ H ₆₀ O ₁₈	805
Rebaudioside B	SVG3	H	Glcβ(1-2)Glcβ(1-3)Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid	58543-17-2	C ₃₈ H ₆₀ O ₁₈	805

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside G	SvG3	Glcβ1-	Glcβ(1-3)Glcβ1	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid(4')-O-β-D-glucopyranosyl ester	127345-21-5	C ₃₈ H ₆₀ O ₁₈	805
Rebaudioside E	SvG4	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(O-β-D-glucopyranosyl-(1,2)-O-[β-D-glucopyranosyl)-oxy]-kaur-16-en-18-oic acid (4')-O-β-D-glucopyranosyl-deoxy-(1,2)-O-[β-D-glucopyranosyl ester	63279-14-1	C ₄₄ H ₇₀ O ₂₃	967
Rebaudioside A	SvG4	Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	58543-16-1	C ₄₄ H ₇₀ O ₂₃	967
Rebaudioside A2	SvG4	Glcβ1-	Glcβ(1-6)[Glcβ(1-2)]Glcβ1-	13-[(6-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester	1326217-29-1	C ₄₄ H ₇₀ O ₂₃	967
Rebaudioside D	SvG5	Glcβ(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	63279-13-0	C ₅₀ H ₈₀ O ₂₈	1129
Rebaudioside L	SvG5	Glcβ1-	Glcβ(1-6)Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(6-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl-3-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester	1220616-38-5	C ₅₀ H ₈₀ O ₂₈	1129

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Steviolmonoside	SVG1	H	Glcβ1-	13-[(β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	60129-60-4	C ₂₆ H ₄₀ O ₈	481
Steviolmonoside A	SVG1	Glcβ1-	H	13-[(hydroxy)kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64977-89-5	C ₂₆ H ₄₀ O ₈	481
Rubusoside	SVG2	Glcβ1-	Glcβ1-	13-[(β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64849-39-4	C ₃₂ H ₅₀ O ₁₃	643
Steviolbioside	SVG2	H	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	41093-60-1	C ₃₂ H ₅₀ O ₁₃	643
Stevioside	SVG3	Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	57817-89-7	C ₃₈ H ₆₀ O ₁₈	805
Stevioside A Or Rebaudioside KA	SVG3	Glcβ(1-2)Glcβ1-	Glcβ1-	13-[(2-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid 4)-O-β-D-glucopyranosyl-deoxy-(1,2)-O-[β-(-D-glucopyranosyl ester	127345-20-4	C ₃₈ H ₆₀ O ₁₈	805
Stevioside B	SVG3	Glcβ(1-3)Glcβ1-	Glcβ1-	13-[(2-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, O-β-D-glucopyranosyl-deoxy-(1,3)-O-[β-D-glucopyranosyl ester	-	C ₃₈ H ₆₀ O ₁₈	805
Rebaudioside B	SVG3	H	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	58543-17-2	C ₃₈ H ₆₀ O ₁₈	805

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside I	SvG5	Glcβ(1-3) Glcβ1-	Glcβ(1-2) [Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₈	1129
Rebaudioside I ₂	SvG5	Glcβ1-	Glcα(1-3) Glcβ(1-2) [Glcβ(1-3)]Glcβ1-	13-[(3-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₈	1129
Rebaudioside I ₃	SvG5	[Glcβ(1-2) Glcβ(1-6)]Glcβ1-	Glcβ(1-2) Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-O-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₈	1129
Rebaudioside Q	SvG5	Glcβ1-	Glcα(1-4) Glcβ(1-2) [Glcβ(1-3)]Glcβ1-	13-[(4-O-β-D-glucopyranosyl)-2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₈	1129
Rebaudioside Q ₂	SvG5	[Glcα(1-2) Glcα(1-4)] Glcβ1-	Glcβ(1-2) Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₈	1129
Rebaudioside Q ₃	SvG5	Glcβ1-	Glcα(1-4) Glcβ(1-3) [Glcβ(1-2)]Glcβ1-	13-[(4-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranosyl-2-O-β-D-glucopyranosyl]-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₈	1129

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside M	SVG6	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-(1,2)-O-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-O-β-D-glucopyranosyl-(1,2)-O-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl ester	1220616-44-3	C ₅₆ H ₉₀ O ₃₃	1291
Related SvGn#1		-	-	-	-	C ₂₁ H ₃₀ O ₁₁	458
Related SvGn#2		-	-	-	-	C ₄₀ H ₇₀ O ₂₄	982
Related SvGn#3		-	-	-	-	C ₃₂ H ₅₂ O ₁₅	676
Related SvGn#4		-	-	-	-	C ₅₀ H ₈₀ O ₂₈	1129
Related SvGn#5		-	-	-	-	C ₄₀ H ₇₀ O ₂₄	982
Group 2: Steviol + Rhamnose + Glucose (SVR1Gn)							
Dulcoside A	SVR1G2	Glcβ1-	Rhaα(1-2)Glcβ1-	13-[(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64432-06-0	C ₃₈ H ₆₀ O ₁₇	789
Dulcoside C	SVR1G2	H	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-rhamnopyranosyl-3-β-D-glucopyranosyl-β-D-glucopyranosyl-oxy]kaur-16-en-18-oic acid		C ₃₈ H ₆₀ O ₁₇	789
Rebaudioside C	SVR1G3	Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-α-L-rhamnopyranosyl-3-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	63550-99-2	C ₄₄ H ₇₀ O ₂₂	951

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside C2	SvR1G3	Rhaα(1-2)Glcβ1	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-rhamnopyranosyl-β-D-glucopyranosyl ester	-	C ₄₄ H ₇₀ O ₂₂	951
Rebaudioside N	SvR1G5	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-(1,2)-O-β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-O-2-deoxy-L-rhamnopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	1220616-46-5	C ₅₆ H ₉₀ O ₃₂	1274
Rebaudioside O	SvR1G6	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)oxy] ent-kaur-16-en-19-oic acid-[(2-O-(3-O-β-D-glucopyranosyl-α-L-rhamnopyranosyl)-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl) ester]	1220616-48-7	C ₆₂ H ₁₀₀ O ₃₇	1436
Rebaudioside O2	SvR1G6	Glcβ(1-4*)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(O-β-D-glucopyranosyl-(1,2)-O-β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-O-β-D-glucopyranosyl-(1,4)-O-6-deoxy-L-rhamnopyranosyl-(1,2)-O-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl ester	-	C ₆₂ H ₁₀₀ O ₃₇	1436

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside K	SVR1G4	Glcβ(1-2)Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-rhamnopyranosyl-3-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	1220616-40-9	C ₅₀ H ₈₀ O ₂₇	1112
Rebaudioside S	SVR1G3	Rhaα(1-2)Glcβ1-	Glcα(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, O-2-deoxy-L-rhamnopyranosyl β-D-glucopyranosyl ester	1931085-11-8	C ₄₄ H ₇₀ O ₂₂	951
Rebaudioside K2	SVR1G4	Glcβ(1-6)Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-rhamnopyranosyl-3-O-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₀ O ₂₇	1112
Rebaudioside H	SVR1G4	Glcβ1-	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	13-[(3-O-β-D-glucopyranosyl-2-O-β-D-rhamnopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	1220616-36-3	C ₅₀ H ₈₀ O ₂₇	1112
Rebaudioside J	SVR1G4	Rhaα(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-O-6-deoxy-L-rhamnopyranosyl-β-D-glucopyranosyl ester	1313049-59-0	C ₅₀ H ₈₀ O ₂₇	1112
Group 3: Steviol + Xylose + Glucose (SVX1Gn)							
Stevioside F	SVX1G2	Glcβ1-	Xylβ(1-2)Glcβ1-	13-[(2-O-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	-	C ₃₇ H ₅₉ O ₁₇	775

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside F	SvX1G3	Glcβ1-	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	438045-89-7	C ₄₃ H ₆₈ O ₂₂	937
Rebaudioside F2	SvX1G3	Glcβ1-	Glcβ(1-2)[Xylβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-xylopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	-	C ₄₃ H ₆₈ O ₂₂	937
Rebaudioside F3	SvX1G3	Xylβ(1-6) Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 6-O-β-D-xylopyranosyl-β-D-glucopyranosyl ester	-	C ₄₃ H ₆₈ O ₂₂	937
Rebaudioside R	SvX1G3	Glcβ1-	Glcβ(1-2)[Glcβ1-3]Xylβ1	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-xylopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	1931083-53-2	C ₄₃ H ₆₈ O ₂₂	937
Rebaudioside U2	SvX1G4	Xylβ(1-2*)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₂ O ₂₆	1099
Rebaudioside T	SvX1G4	Xylβ(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-xylopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₂ O ₂₆	1099
Rebaudioside V2	SvX1G5	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₆ H ₉₂ O ₃₁	1261

Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside V	SvX1G5	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	Xylβ(1-2*)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-xylopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	-	C ₅₆ H ₁₀₂ O ₃₁	1261
Group 4: Steviol + Arabinose + Glucose (SvA1Gn)							
Rebaudioside U	SvA1G4	Araα(1-2*)Glcβ1	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]ent-kaur-16-en-19-oic acid-(6-O-α-L-arabinopyranosyl)-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₂ O ₂₆	1098
Rebaudioside W	SvA1G4	Glcβ(1-2)[Araβ(1-3*)]Glcβ1	Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₂ O ₂₆	1098
Rebaudioside W ₂	SvA1G4	Araβ(1-2*)Glcβ1	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₂ O ₂₆	1098
Rebaudioside W ₃	SvA1G4	Araβ(1-6)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 6-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester	-	C ₅₀ H ₈₂ O ₂₆	1098
Rebaudioside Y	SvA1G5	Glcβ(1-2)[Araβ(1-3*)]Glcβ1	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-glucopyranosyl-3-O-β-D-arabinopyranosyl-β-D-glucopyranosyl ester	-	C ₅₆ H ₁₀₂ O ₃₁	1260

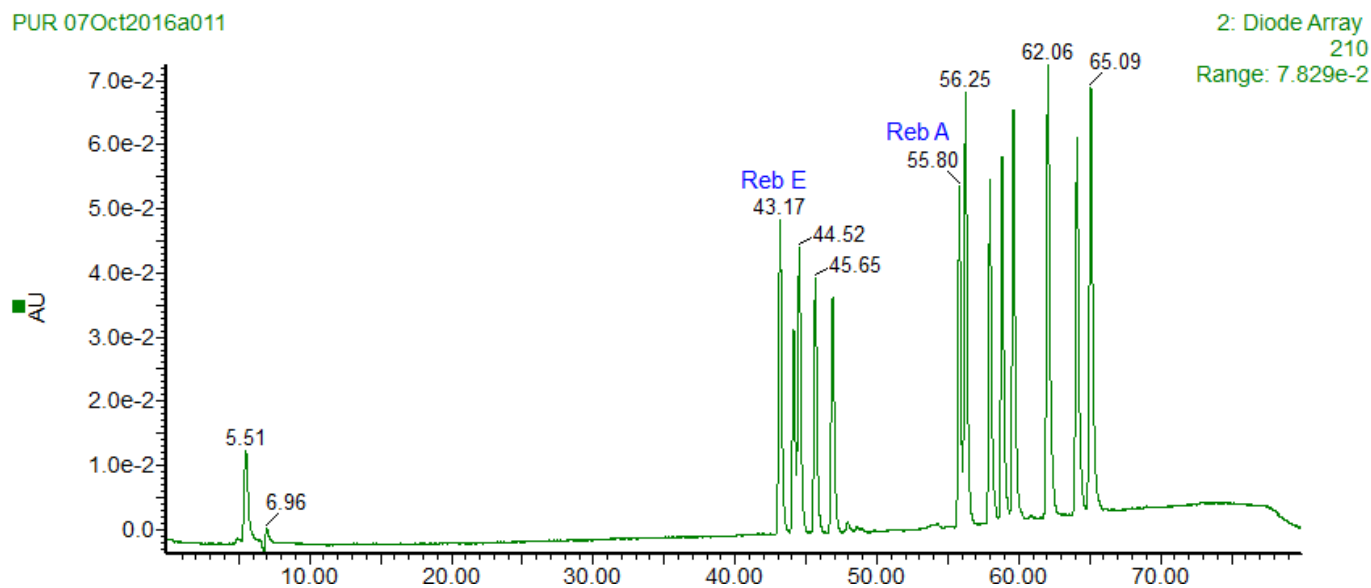
Common Name	Trivial Name	R ₁	R ₂	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Group 5: Steviol + Galactose + Glucose (SvGa1Gn)							
Rebaudioside T1	SvGa1G4	Galβ(1-2*)Glcβ1	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, 2-O-β-D-galactopyranosyl-β-D-glucopyranosyl ester	-	C50H80O28	1128
Group 6: Steviol + Fructose + Glucose (SvFruGn)							
Rebaudioside A3	SbF1G3	Glcβ1-	Glcβ(1-2)[Fruβ(1-3)]Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-fructofuranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	-	C44H70O22	951
Group 7: Steviol + -de-oxy glucose + Glucose (SvdG1Gn)							
Stevioside D	SvDg1G2	Glcβ1-	6-deoxy Glcβ(1-2)Glcβ1-	13-[(2-O-β-D-6-deoxyglucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	-	C38H60O17	789
Stevioside E	SvDg1G3	Glcβ1-	6-deoxy Glcβ(1-2)[Glcβ(1-3)] Glcβ1-	13-[(2-O-β-D-6-deoxyglucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	-	C44H70O22	951
Stevioside E2	SvDg1G3	6-deoxy Glcβ1-	Glcβ(1-2)[Glcβ(1-3)] Glcβ1-	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]kaur-16-en-18-oic acid, β-D-6-deoxyglucopyranosyl-ester	-	C44H70O22	951

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.

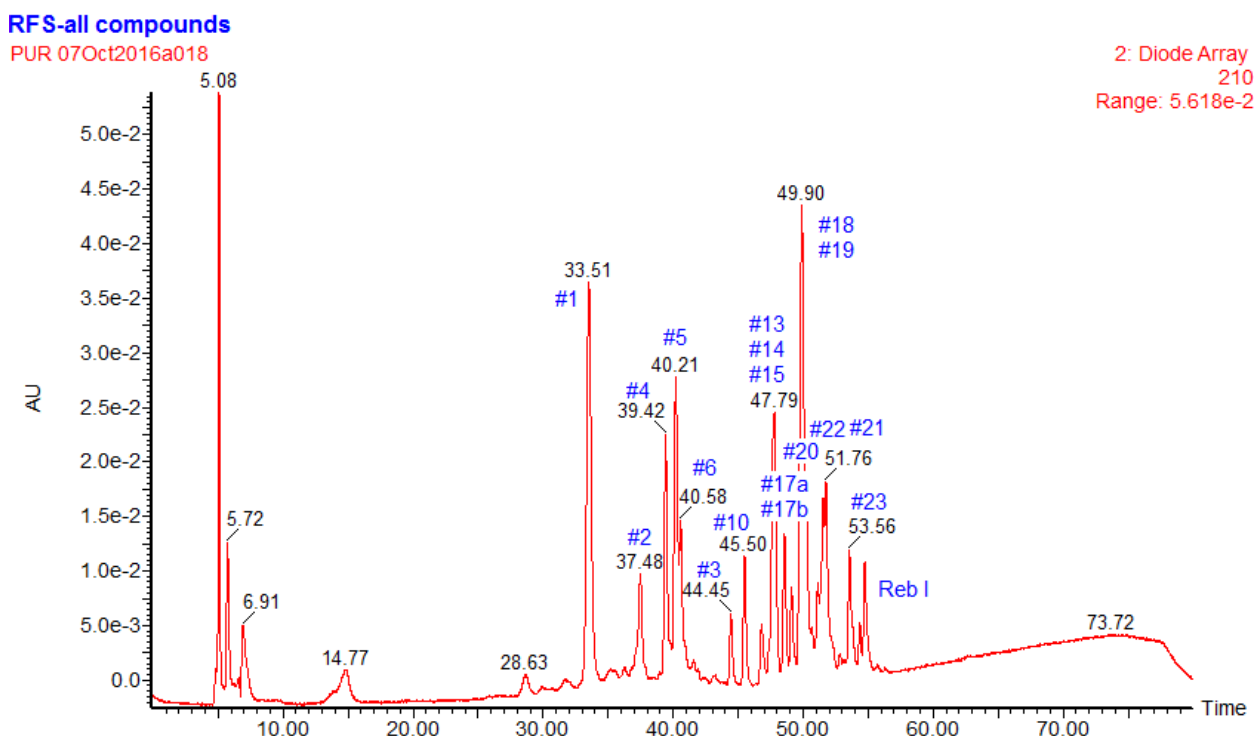
Glc, Rha, Fru, deoxyGlc, Gal, Ara and Xyl represent, respectively, glucose, rhamnose, fructose, deoxyglucose, galactose, arabinose and xylose sugar moieties.

Note: This list is not exhaustive. More steviol glycosides may have been identified in stevia leaf extracts in the literature

Appendix- 2: Representative chromatograms for steviol glycosides using Method of Assay



Example Chromatogram of Representative Steviol Glycoside Standards from a Phenomenex Luna C18 (150 mm x 4.6 mm, 5 μ m). Order of retention times from left to right: rebaudioside E, rebaudioside O, rebaudioside D, rebaudioside N, rebaudioside M, rebaudioside A, stevioside, rebaudioside F, rebaudioside C, dulcoside A, rubusoside, rebaudioside B and steviolbioside.



Example Chromatogram from a Phenomenex Luna C18 (150 mm x 4.6 mm, 5 μ m) of Minor Steviol Glycosides using in-house purified reference standards.

Appendix-3: Typical Retention Time (RT), Relative Retention Time (RRT) and Mass Ions of Steviol Glycosides

Compound Name	Typical Retention Time (RT)*	Relative Retention Time to Rebaudioside A (RRT)*	Molecular Mass Ion [M-H]
Related steviol glycoside #1	32.6	0.58	517 or 427
Related steviol glycoside #2	33.6	0.60	981
Related steviol glycoside #3	34.3	0.61	427 or 735
Related steviol glycoside #4	38.1	0.68	675 or 1127
Related steviol glycoside #5	40.8	0.73	981
Rebaudioside V	43.0	0.77	1259
Rebaudioside T	42.0	0.75	1127
Rebaudioside E	43.7	0.78	965
Rebaudioside O	44.6	0.79	1435
Rebaudioside D	45.1	0.80	1127
Rebaudioside K	45.8	0.81	1111
Rebaudioside N	46.1	0.82	1273
Rebaudioside M	47.5	0.84	1289
Rebaudioside S	48.3	0.86	949
Rebaudioside J	48.4	0.86	1111
Rebaudioside W	49.1	0.87	1097
Rebaudioside U2	49.1	0.87	1097
Rebaudioside W2	49.7	0.88	1097
Rebaudioside W3	50.3	0.89	1097
Rebaudioside U	50.7	0.90	1097
Rebaudioside O2	50.6	0.90	965
Rebaudioside Y	50.8	0.90	1259
Rebaudioside I	50.7	0.90	1127
Rebaudioside V2	52.2	0.93	1259
Rebaudioside K2	51.7	0.93	1111
Rebaudioside H	53.7	0.96	1111
Rebaudioside A	56.2	1.00	965
Stevioside	56.6	1.01	803
Rebaudioside F	58.3	1.04	935
Rebaudioside C	59.2	1.05	949
Dulcoside A	60.0	1.07	787
Rubusoside	62.4	1.11	641
Rebaudioside B	64.5	1.15	803
Steviolbioside	65.5	1.17	641

*RT and RRT values given in the above table are for information purpose only. They may vary based on the chromatographic system and conditions used. Analyst needs to establish during method validation.

SUCROSE ESTERS OF FATTY ACIDS

Prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017), superseding specifications prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010). An ADI of 0 - 30 mg/kg bw for this substance together with sucroglycerides, sucrose oligoesters type I and type II and sucrose monoesters of lauric, palmitic or stearic acid was established at the 73rd JECFA (2010).

SYNONYMS

Sucrose fatty acid esters, INS No. 473

DEFINITION

Mono-, di- and tri-esters of sucrose with food fatty acids, prepared from sucrose and methyl and ethyl esters of food fatty acids by esterification in the presence of a catalyst or by extraction from sucroglycerides. Only the following solvents may be used for the production: dimethylformamide, dimethyl sulfoxide, ethyl acetate, isopropanol, propylene glycol, isobutanol and methyl ethyl ketone.

Assay

Not less than 80% of sucrose esters

DESCRIPTION

White to greyish white or pale yellow powder, stiff gel or soft solid

FUNCTIONAL USES

Emulsifier

CHARACTERISTICS**IDENTIFICATION**Solubility (Vol.4)

Very soluble in ethanol at 50°

Fatty acids

Add 1 mL of ethanol to 0.1 g of the sample, dissolve by warming, add 5 mL of dilute sulfuric acid TS, heat in a water bath for 30 min and cool. A yellowish white solid or oil is formed, which has no odour of isobutyric acid, and which dissolves when 3 mL of diethyl ether are added. Use the aqueous layer separated from the diethyl ether in the Test for sugars.

Sugars

To 2 mL of the aqueous layer separated from the diethyl ether in the test for fatty acids, carefully add 1 mL of anthrone TS down the inside of a test tube; the boundary surface of the two layers turns blue or green.

PURITYSulfated ash (Vol.4)

Not more than 2%
Test 1 g of the sample (Method I)

<u>Acid value</u> (Vol.4)	Not more than 6
<u>Free sucrose</u>	Not more than 5% See description under TESTS
<u>Dimethylformamide</u>	Not more than 1 mg/kg See description under TESTS
<u>Dimethyl sulfoxide</u>	Not more than 2 mg/kg See description under TESTS
<u>Ethyl acetate, isopropanol and propylene glycol</u>	Not more than 350 mg/kg, singly or in combination See description under TESTS
<u>Isobutanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methyl ethyl ketone</u>	Not more than 10 mg/kg See description under TESTS
<u>Lead</u> (Vol.4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique 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

<u>Free sucrose</u>	Determine by gas chromatography (Vol. 4) under the following conditions: Standard solutions Prepare a stock solution containing 5.0 mg/mL of sucrose in N,N-dimethylformamide. Prepare a range of standard solutions containing 0.5, 1.25 and 2.5 mg/mL of sucrose by dilutions of the stock solution with N,N-dimethylformamide. Internal standard solution Weigh accurately 0.25 g of octacosane into a 50-mL volumetric flask, add 25 mL of tetrahydrofuran to dissolve the octacosane, and add tetrahydrofuran to the mark. Chromatography conditions <ul style="list-style-type: none"> - Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film) - Carrier gas: Helium
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- Flow rate: 1.5 mL/min
- Detector: Flame-ionization detector (FID)
- Temperatures:
 - o injection port: 280°
 - o column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
 - o detector: 320°

The retention times of free sucrose and octacosane measured under the above conditions are approx. 18.8 and 19.3 min, respectively.

Procedure:

Weigh accurately 20-50 mg of the sample into a centrifugation tube, add 1 mL internal standard solution, 1 mL N,N-dimethylformamide, 0.4 mL of N,O-bis(trimethylsilyl)acetamide (BSA) and 0.2 mL trimethylchlorosilane (TMCS). After sealing the tube, shake and let stand for 5 min at room temperature. Inject 1 µL into the chromatograph.

Standard curve

Prepare silylated standard solutions following the above procedure using 1 mL each of the standard solutions in place of the sample and N,N-dimethylformamide. Draw a standard curve by plotting amount of sucrose (mg) in 1 mL of the standard solution (X-axis) vs. ratio of peak area of sucrose/internal standard (Y-axis).

Measure the peak areas for sucrose and internal standard.

Calculate the ratio of their peak areas, and obtain the amount of sucrose from the standard curve.

Calculate the percentage of free sucrose from:

$$\% \text{ free sucrose} = \frac{\text{amount of sucrose determined (mg)}}{\text{weight of sample (mg)}} \times 100$$

Dimethylformamide

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

Standard solutions

Prepare a stock solution containing 1.00 mg/mL of dimethylformamide in tetrahydrofuran. Prepare a range of standard solutions containing 0.05, 0.1 and 0.2 µg/mL of dimethylformamide by diluting the stock solution with tetrahydrofuran.

Chromatography conditions

- Column: Polyethylene glycol (30 m x 0.32 mm i.d. with a 0.5 µm film)
- Carrier gas: Helium
- Pressure: 150 kPa (constant pressure)

- Detector: Nitrogen/phosphorus detector or thermionic specific detector)
- Temperatures:
 - o injection port: 180°
 - o column: Hold for 2 min at 40°, then 40-160° at 20°/min, hold for 2 min at 160°
 - o detector: 325°
- Injection method: Splitless injection of 1.0 µl with auto-injector, followed by start of purge after 1.0 min.

The retention time of dimethylformamide measured under the above conditions is approx. 6.4 min.

Procedure

Weigh accurately 2 g of sample into a 20-mL volumetric flask, add 10 mL of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 1.0 µl of the sample solution into the chromatograph.

Standard curve

Prepare daily by injecting 1.0 µl of each of the standard solutions into the chromatograph.

Calculate the concentration of dimethylformamide in mg/kg (CDFA) from:

$$CDFA (mg/kg) = C \times 20/W$$

where

- C is dimethylformamide concentration determined (µg/mL);
- W is weight of sample (g)

Note: The column must be reconditioned frequently. Overnight reconditioning (flow carrier gas in the reverse direction at 180° without the connection of the detector) is required after about every 15 samples.

Dimethyl sulfoxide

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

Standard solutions

Prepare a 0.25 mg/mL stock solution of dimethyl sulfoxide in tetrahydrofuran. Prepare a range of solutions containing 0.1, 0.2, 0.4 and 1.0 µg/mL of dimethyl sulfoxide by dilutions of the stock solution with tetrahydrofuran.

Chromatography conditions

- Column: 10% PEG 20M and 3% KOH on Chromosorb W AW DMCS 60/80 mesh (2 m x 3 mm i.d.) or equivalent.
- Raise the oven temperature to 180° at a rate of 10°/min and let stabilize for 24 to 48 h with 30 to 40 mL/min of nitrogen for conditioning
- Carrier gas: Nitrogen
- Flow rate: 30 mL/min

- Detector: Flame photometric detector (using 394 nm sulfur filter)
- Temperatures
 - o injection port: 210°
 - o column: 160°

The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3.4 min.

Procedure

Weigh accurately 5 g of the sample into a 25-mL volumetric flask, add 10 mL of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 3 µl of the sample solution into the chromatograph..

Standard curve

Prepare daily by injecting 3 µl of each of the standard solutions into the chromatograph.

Calculate the concentration of dimethyl sulfoxide in mg/kg (CDMSO) from:

$$CDMSO (mg/kg) = C \times 25/W$$

where

- C is dimethyl sulfoxide concentration determined (µg/mL);
- W is weight of sample (g).

Propylene glycol (Vol. 4)

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 with 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:
 - o injection port: 230°
 - o column: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
 - o detector: 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 the 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 the 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 (CPG) from:

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

where

- C is polyethylene glycol concentration determined (µg/mL);
- W is weight of sample (g).

Methanol, isopropanol, isobutanol, ethyl acetate and methyl ethyl ketone

Determined by gas chromatography (vol. 4) with a head space sampler under the following conditions.

Standard solutions

Prepare standard solution A containing 4000 mg/L each of methanol, isopropanol, isobutanol, ethyl acetate and methyl ethyl ketone by weighing accurately 0.2 g of each solvent into a 50-mL volumetric flask containing approx. 20 mL of water, then adding water to volume. By dilutions of this solution, prepare solutions containing 2000 mg/L (standard solution B) and 1000 mg/L (standard solution C).

Procedure

Weigh accurately 1 g of the sample into each of four sample vials. To one vial add 5 µl of water, to the second, third and fourth, add, respectively, standard solutions A, B and C, and seal them quickly with a septum. (The concentrations of each solvent after adding 5 µl of standard solutions A, B and C to 1g of the sample are equal to 20, 10 and 5 mg/kg of sample, respectively). Place the sample vials in a head space sampler and analyse using the following conditions:

- Column: 100% Polydimethylsiloxane (30 m x 0.53 mm i.d. with 1.5 µm film:
- Carrier gas: Nitrogen
- Flow rate: 3.5 mL/min
- Detector: FID
 - o Temperatures
 - o injection port: 110°
 - o column: 40°
 - o detector: 110°

- Head space sampler:
 - o sample heat insulating temperature: 80°
 - o sample heat insulating period: 40 min
 - o syringe temperature: 85°
 - o sample gas injection: 1.0 mL

Calculation

Plot the relationship between the added amounts against the peak area for each solvent using the analytical results. The relationship should be linear. Extrapolate and determine the x-intercept (w_i), and calculate the solvent concentrations (C_i) in the sample from:

$$C_i(\text{mg/kg}) = w_i/W$$

where

- w_i is x-intercept of relationship line using the standard addition method (μg)
- W is weight of sample (g)

METHOD OF ASSAY

Determine by HPLC (Vol. 4) under the following conditions.

Procedure

Accurately weigh 250 mg of the sample into a 50-mL volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.45 μm membrane filter. Inject 80 μl of the sample solution into the pre-stabilized chromatograph.

Chromatography conditions

- Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G1000HXL, G2000HXL, G3000HXL, G4000HXL (each 30 cm x 7.8 mm i.d., 5 μm in series), Tosoh Co. or equivalent)
- Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 0.8 mL/min
- Detector: Refractive index
- Temperatures:
 - o Column: 40°
 - o Detector: 40°
- Record the chromatogram for about 50 min.

Typical retention times under the above conditions are described in Table 1. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 1. Typical retention time (min) of mono-, di- and tri-esters esterified with main fatty acids

Esterified fatty acid	Mono-esters	Di-esters	Tri-esters
Lauric acid	40.0	38.2	37.0

Palmitic acid	39.3	37.2	36.0
Stearic acid	39.0	37.0	35.7
Oleic acid	39.1	37.1	35.9

Calculate the percentage of sucrose ester content in the sample from:

$$\% \text{ sucrose ester} = 100 A/T$$

Where

- A is the sum of peak areas for the three main components, the mono-, di- and tri-esters;
- T is the sum of all peak areas eluting within 43 min

TAMARIND SEED POLYSACCHARIDE

New specifications prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017). An ADI of 'not specified' was established at the 84th JECFA (2017).

SYNONYMS

Tamarind seed gum, tamarind gum, tamarind xyloglucan, tamarind seed xyloglucan, tamarind galactoxyloglucan

DEFINITION

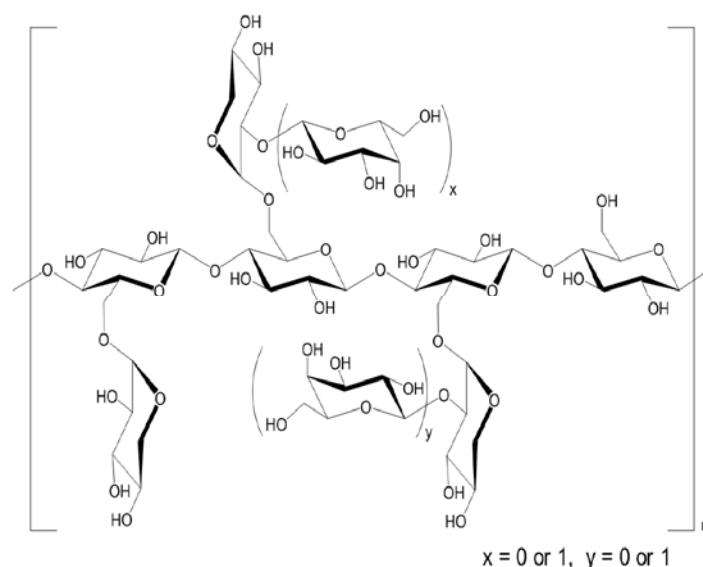
Tamarind seed polysaccharide is a high molecular weight (400-6000 kDa) polysaccharide. It is produced from the kernel of tamarind seed by husking and pulverizing the seeds of *Tamarindus indica* L. It is composed of a linear chain of D-glucose units linked by $\beta(1-4)$ glycosidic bonds. Single D-xylose units are attached to about 75% of these D-glucose units via $\alpha(1-6)$ bonds. Single D-galactose units are attached to some of the D-xylose units through $\beta(1-2)$ bonds. The molar ratio of D-glucose:D-xylose:D-galactose is about 4:3:1.

Tamarind seed polysaccharide is obtained from tamarind kernel powder by treating it with an aqueous solution of methanol, followed by sodium hydroxide or sulphuric acid to wash and adjust pH. The insoluble polysaccharide is separated from the supernatant (containing protein, fat and minerals) by centrifuging. The material is dried, pulverized, sieved and mixed with food-grade bulking agents to standardize the product. Depending on the pH adjustment, and further alkali treatment and/or purification with methanol or 2-propanol, tamarind seed polysaccharide products with varying viscosity can be manufactured.

C.A.S. Number

39386-78-2

Structural formula



Assay

Not less than 75% on the dried basis

DESCRIPTION	White to light brown powder, nearly odourless
FUNCTIONAL USES	Thickener, stabilizer, emulsifier and gelling agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in hot water (75°); insoluble in ethanol

Precipitate and Colour Formation Passes the tests.
See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 14.0% (105°, 5 h)

Ash (Total) (Vol. 4) Not more than 1.0% on the dried basis

Protein (Vol. 4) Not more than 3.0%
Weigh accurately 1.0 g tamarind seed polysaccharide, and proceed as directed under Nitrogen determination (Kjeldahl Method, Method 1) in Volume 4 (under "General Methods, Inorganic components"). Multiply %N with 6.25 to get % protein.

Residual solvents (Vol. 4) Methanol: Not more than 200 mg/kg
2-propanol: Not more than 1,000 mg/kg

See description under TESTS.

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 under Metallic Impurities in Volume 4 (under "General Methods, Inorganic components").

Microbiological criteria (Vol. 4) Total plate count: Not more than 5,000 cfu/g
Escherichia coli: Negative in 1 g
Salmonella: Negative in 5 g
Yeasts & moulds: Not more than 500 cfu/g

TESTS

IDENTIFICATION TEST

Precipitate and colour formation (Vol. 4) Reagents:
- Sodium hydroxide
- Iodine TS

- Sodium sulfate

Sodium hydroxide solution

Dissolve 22 g of sodium hydroxide in water, and dilute up to 1000 mL with deionized water in a volumetric flask. Store in a polyethylene bottle.

Sodium sulfate (saturated)

Dissolve 20 g of sodium sulfate and make up to 100 mL of deionized water in a volumetric flask. Store in a polyethylene bottle.

Preparation of sample solution (20 mg/mL)

Add gradually 2 g of Tamarind Seed Polysaccharide sample to 100 mL of sodium hydroxide solution, and dissolve by vigorous stirring.

To 5 mL of the sample solution, add 3 mL of saturated sodium sulfate. White lumps are produced. Observe and record.

To 5 mL of the sample solution, add a few drops of Iodine TS. Dark blue-green lumps are produced on the solution surface, and the colour disappears on stirring. Observe and record.

PURITY TESTS

Residual solvents (Vol. 4) Determine by the method based on headspace gas chromatography as directed under Residual solvents in Volume 4 (under "General Methods, Organic components"). Prepare sample as follows:

Sample solution:

Weigh accurately 0.12 g sample into a headspace vial. Add 5.0 mL water and add 1.0 mL of the internal standard solution, to obtain a final sample concentration of 2%.

METHOD OF ASSAY

Principle

The assay is based on a solution of xyloglucan reacting with iodine to give a specific greenish color, the intensity of which depends on the concentration of xyloglucan.

Reagents

- Iodine: purity $\geq 99.8\%$
- Potassium iodide: purity $\geq 99.5\%$
- Sodium sulfate: purity $\geq 99.0\%$
- In-house Reference Standard (Not commercially available; prepare following the procedure below)

Preparation of Solutions

Preparation of 0.5% w/v Iodine-1.0% w/v Potassium iodide solution

Place 0.50 g iodine and 1.0 g potassium iodide in a 100 mL glass beaker. Add 75 mL deionized water and put a stir bar to the

beaker. Cover the beaker with plastic wrap and then with aluminium foil to protect from light. Stir the mixture for 2 h with a magnetic stirrer. Stir for another 30 min and check for complete dissolution. Transfer to a 100 mL volumetric flask and make up to volume with deionized water. Stir to obtain a homogeneous solution. Label as 0.5 w/v% Iodine-1.0 w/v% Potassium Iodide aqueous solution. Store the solution in a refrigerator, and away from light. The solution is stable in a refrigerator, in darkness for several months.

Sodium sulfate (15% w/v) solution

Place 800 mL deionized water in a 1 L glass beaker, put a stir bar to the beaker, and add 150.0 g sodium sulfate while stirring. Continue stirring until all the sodium sulfate has dissolved. Transfer to a 1000 mL volumetric flask and make up to volume with deionized water. Stir to obtain a homogeneous solution. Label as Sodium sulfate (15% w/v) solution. Store the solution at room temperature.

Preparation of In-house Reference Standard

Prepare a 0.5% w/v solution of a reference tamarind seed polysaccharide that has been filtered and alkali treated. Centrifuge to remove impurities, if any. Precipitate by adding 2-propanol. Filter, wash the precipitate with 2-propanol. Repeat precipitation and filtration step. Dry and grind. Label as "In House Reference Standard". Store in house reference standard in a desiccator.

Preparation of Sample

Place 170 mL deionized water at room temperature in a beaker. Weigh exactly 1.0 g sample (separately measure its loss on drying[%]), disperse in deionized water while stirring, and dissolve by stirring at room temperature for 15 min. Adjust this solution to exactly 200 g with deionized water and stir to obtain a homogeneous solution. Weigh exactly 1.0 g of this solution; add deionized water to exactly 50.0 g and mix to make the solution homogeneous. Prepare before use in assay.

Preparation of Standard Solutions

Place 170 mL deionized water at room temperature in a beaker. Weigh exactly 1.0 g in-house reference standard. Disperse in deionized water while stirring, and dissolve by stirring at room temperature for 15 min. Adjust this solution to exactly 200 g with deionized water and stir to obtain a homogeneous solution. Weigh exactly 4.0 g of this solution; add deionized water to exactly 40.0 g and mix to obtain a homogeneous solution.

Weigh exactly 2.0, 3.0, 4.0, 5.0 and 6.0 g of this solution; add deionized water to exactly 20.0 g and mix to obtain a homogeneous solution. Use the resulting solutions as 0.005, 0.0075, 0.01, 0.0125 and 0.015 w/w% (equivalent to 50, 75, 100, 125, and 150 µg/g respectively) standard solutions, respectively, which should be prepared before use. Calculate the actual concentrations of the in-house reference standard (on a dry matter basis) in each solution prepared.

Procedure

Add 1 mL each of deionized water (blank), sample or standard solutions to test tubes. Add 2 mL of 15% w/v Sodium Sulfate Aqueous Solution and 0.25 mL of 0.5% w/v Iodine–1.0% w/v Potassium Iodide Aqueous Solution to each test tube. Prepare duplicates for each standard, sample, and blank.

After vigorous mixing for a few seconds, close the test tube with a stopper. Place the test tube in a refrigerator and in the dark immediately. Allow to stand for not less than 1 hour to fix Iodine, and then for another 30 min in the dark at room temperature. Transfer the blank to a cuvette, mount on a spectrophotometer and use to set the absorbance at 640 nm to zero. Transfer the prepared sample and standard solutions to cuvettes to measure the absorbance of each at 640 nm.

Produce a standard curve using Absorbance vs. Concentration of in-house reference standard using the values obtained for the standard solutions, where the zero point is excluded with a coefficient of correlation of at least 0.99. If the coefficient of correlation is below 0.99, repeat all preparations of standard solutions to generate a new standard curve. Deduce the concentration of tamarind polysaccharide in the sample from the standard curve.

$$T = \frac{D}{W_T \times \left(\frac{100 - L}{100}\right)}$$

Where

- T is %Tamarind Seed Polysaccharide in the sample
- D is concentration of Tamarind Seed Polysaccharide determined in the sample solution, $\mu\text{g/g}$
- W_T is weight of the sample, g
- L is percent loss on drying of the sample

YEAST EXTRACTS CONTAINING MANNOPROTEINS (TENTATIVE)

New specifications prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017). No ADI was established at the 84th JECFA (2017) as the use of this substance is not of health concern when used for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.

Information required on:

- *composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;*
- *analytical data from five batches of each commercial product, including information related to impurities; and*
- *data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.*

*The Committee requests that this information be submitted by **December 2018**.*

SYNONYMS

INS.No. 455

DEFINITION

Yeast extracts containing mannoprotein represents a large family of natural compounds from yeast (*Saccharomyces cerevisiae*) in which polysaccharide chains are bound to proteins and peptides by covalent and non-covalent bonds (i.e., ionic interactions). The structures and molecular weights of mannoprotein vary, depending on the degree and type of glycosylation. The polysaccharide chains consist almost exclusively of mannose units linked together by α -links, with a long α -1 \rightarrow 6 linked backbone containing short α -1 \rightarrow 2- and α -1 \rightarrow 3 linked side chains. Several of the side chains may have phosphodiester linkages to other mannosyl residues. Yeast mannoproteins are extracted from purified yeast cell walls by enzymatic extraction using glucan 1,3- β -glucosidase (EC 3.2.1.58) or by thermal treatment extraction. The enzyme hydrolyses the yeast cell wall allowing the mannoproteins to be solubilized. The thermal treatment breaks the links with β -glucans. The mannoproteins thus solubilized by either treatment are then separated from the insoluble cell wall material, concentrated and micro- or ultra-filtered. Mannoproteins have molecular weights ranging from 20 kDa to more than 450 kDa.

Assay

Total polysaccharides: Not less than 60% on dried basis

Mannose: Not less than 70% of the total polysaccharides

Nitrogen content: 0.5-7.5% on the dried basis

DESCRIPTION

White or beige, odourless powder, or yellow, translucent colloidal solution

FUNCTIONAL USES Stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water and insoluble in ethanol

PURITY

Loss on drying (Vol.4) Powder form: Not more than 15% (105°, 5h)

Specific rotation (Vol. 4) $[\alpha]_D^{20}$: between +80 and +150°,
Test solution: 1.0 g of dried sample in 100 mL of water, using an optical cell with 100-mm path length.

Total Ash (Vol. 4) Not more than 8%, on dried basis

Lead (Vol. 4) Not more than 2 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 methods described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria (Vol. 4) Aerobic plate count: Not more than 10,000 CFU/g

Coliforms: Not more than 10 CFU/g

Staphylococcus aureus: Negative in 1 g

E. coli: Negative in 25 g

Salmonella: Negative in 25 g

Moulds: Not more than 50 CFU/g

Yeasts: Not more than 100 CFU/g

METHOD OF ASSAY

Total polysaccharides

Reagents:

- Mannose, >99 % pure
- Sulfuric acid, Concentrated
- Phenol solution (50 mg/mL): Dissolve 5 g of phenol in 100 mL of deionized water

Preparation of mannose standard solution (0.1 mg/mL): Accurately weigh 100 mg of mannose, dissolve in deionized water and make up to 100 mL in a volumetric flask. Pipette 5 mL of solution into a 50 mL volumetric flask and make up to volume with deionized water (0.1 mg/mL).

Preparation of sample solution (15 mg/L): Accurately weigh 150 mg (W) of sample, dissolve in deionized water and make up to 100 mL in a volumetric flask. Pipette 1 mL of solution into a 100 mL volumetric flask and make up to volume with deionized water (15 mg/L).

Procedure:

Add 200 µl of phenol solution and 1 mL of concentrated sulfuric acid to 200 µl of the sample solution and mix immediately. Prepare a reference solution by adding 200 µl of phenol solution and 1 mL of concentrated sulphuric acid to 200 µl of mannose standard solution and mix immediately. Heat both solutions to 100° in a water bath for 5 min, remove tubes and quickly cool to 0° in ice. Take out the tubes from ice and allow tubes to reach room temperature. Measure the absorbance values at 490 nm in a spectrophotometer against a blank solution prepared similarly omitting the standard.

Calculation

Calculate the amount of total polysaccharides according to:

$$\text{Total polysaccharides} = \frac{A_{\text{Sample}} \times 10^7}{A_{\text{Std}} \times W \times (100 - \%M)}$$

Where:

- Total polysaccharides are given in %w/w on dried basis
- A_{Sample} is the absorbance of the sample solution
- A_{Std} is the absorbance of the standard solution (0.1 mg/mL)
- W is the weight of sample, mg
- %M is the loss on drying, %

Mannose

Instrumentation and reagents:

- Spectrophotometer: 340 nm
- Stop-watch
- Triethanolamine hydrochloride ($\text{C}_6\text{H}_5\text{NO}_3 \cdot \text{HCl}$): >99 % pure
- Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$): AR grade
- Sodium hydroxide: AR grade
- Disodium nicotinamide adenine dinucleotide phosphate: AR grade
- Adenosine-5'-triphosphate (ATP): AR grade
- Sodium hydrogen carbonate: AR grade
- Hexokinase solution: 2 mg of protein/mL or 280 U/mL
- Glucose-6-phosphate(G-6-P)-dehydrogenase solution: 1 mg of protein/mL
- Phosphoglucose-isomerase (PGI) solution: 2 mg of protein/mL or 700 U/mL
- Phosphomannose isomerase solution: 616 U/mL
- Sulfuric acid: 5 M
- Potassium hydroxide: 10 M

Buffer solution (0.3 M triethanolamine, 0.004 M Mg^{2+} , pH 7.6):
Dissolve 11.2 g of triethanolamine hydrochloride and 0.2 g magnesium sulfate in 150 mL deionized water, adjust the pH 7.6 with about 4 mL of 5 mol/L sodium hydroxide solution and make up to 200 mL.

Nicotinamide adenine dinucleotide phosphate (NADP) solution (10 mg/mL, about 0.012M):
Dissolve 50 mg disodium nicotinamide adenine dinucleotide phosphate in 5 mL of deionized water.

Adenosine-5'-triphosphate (ATP) solution (50 mg/mL, about 0.08 M):
Dissolve 250 mg disodium adenosine-5'-triphosphate and 250 mg sodium hydrogen carbonate in 5 mL of deionized water.

Hexokinase/glucose-6-phosphate(G-6-P)-dehydrogenase solution:
Mix 0.5 mL hexokinase solution with 0.5 mL G-6-P-dehydrogenase solution.

Preparation of sample solution (5 mg/mL):
Accurately weigh 500 mg (W) of sample, dissolve in 100 mL of deionized water. Place 100 μ L of the sample solution in airtight sealed tubes and add 1 mL of 5M sulphuric acid solution. Cap the tubes, heat at 100° in a water bath for 30 min, remove tubes and quickly cool to 0° in ice. Take out the tubes from ice and allow tubes to reach room temperature. Neutralise the acid by adding 1 mL of 10 M potassium hydroxide solution to each tube.

Procedure:
Set the spectrophotometer at 340 nm wavelength. Using matched cells, zero the instrument (according to the manufacturer's instructions),

Prepare the reference cell with 1 cm path length with 2.50 mL buffer solution (@20°), 0.10 mL NADP solution, 0.10 mL ATP solution and 0.20 mL deionised water

Prepare the sample cell like the reference cell by replacing the deionised water with 0.20 mL of sample solution

Start the stop-watch and mix the solution in the cell. Add 0.02 mL of G-6-P-dehydrogenase solution to both cells after three minutes and mix. Add 0.02 mL of PGI Solution to both cells after 17 min and mix. Read the absorbance of the solution in reference as well as sample cells, after 10 min. After two more minutes, read the absorbance (A_1) of the solution to ensure that the reaction has stopped (indicated by no increase in absorbance).

Add 0.02 mL each of phosphomannose isomerase solution (616 U/mL) and mix. Read the absorbance after 30 min. Check absorbance (A_2) after two more minutes to ensure that the reaction has stopped (indicated by no increase in absorbance).

Note: The time needed for the completion of enzyme activity may vary from one batch to another. The above value is given only for

guidance and it is recommended that it be determined for each batch.

Calculation:

Calculate the differences in absorbance between A_1 and A_2 for the reference cell (ΔA_T) and the sample cell (ΔA_D), and then obtain $\Delta A_M = \Delta A_D - \Delta A_T$

Calculate mannose %w/w (on dried basis) by the following expression:

$$\text{Mannose} = \frac{0.423 \times \Delta A_M \times 10^6 \times 21}{W \times (100 - \%M)}$$

Where:

- Mannose is expressed as % w/w on dried basis
- W is the weight of sample, mg
- %M is the loss on drying, %

Calculate the % Mannose in total polysaccharides as follows:

$$\% \text{ Mannose in total polysaccharides} = \frac{\% \text{Mannose on dried basis} \times 100}{\% \text{ Polysaccharide on dried basis}}$$

Nitrogen Content

Weigh accurately 1.0 g of yeast mannoprotein, and proceed as directed under Nitrogen determination (Kjeldahl Method, Method 1) in Volume 4 (under "General Methods, Inorganic components").

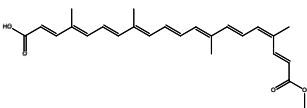
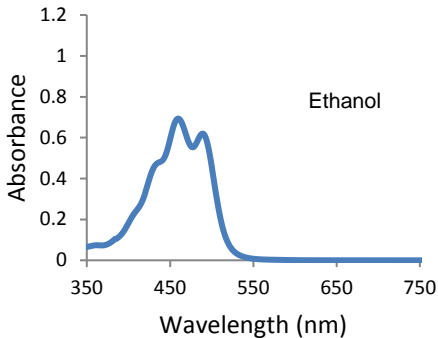
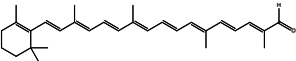
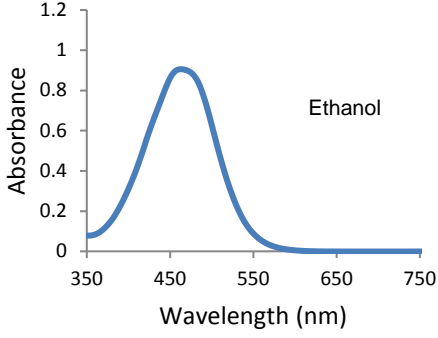
ANALYTICAL METHODS

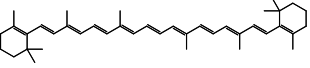
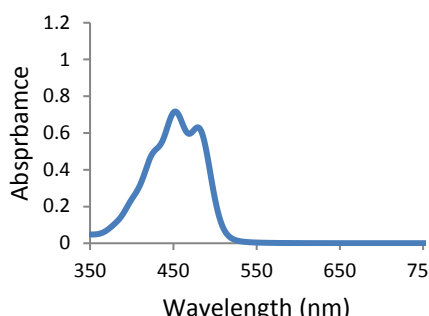
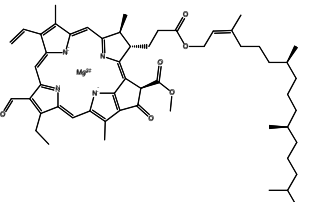
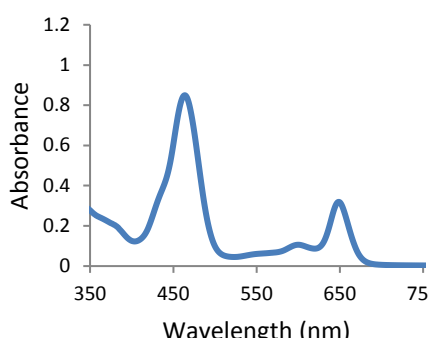
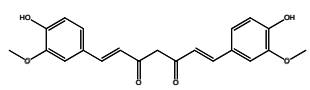
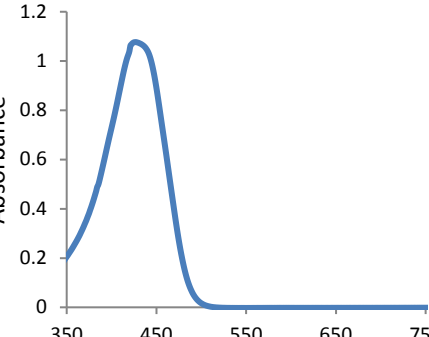
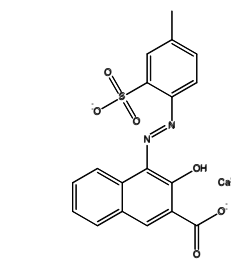
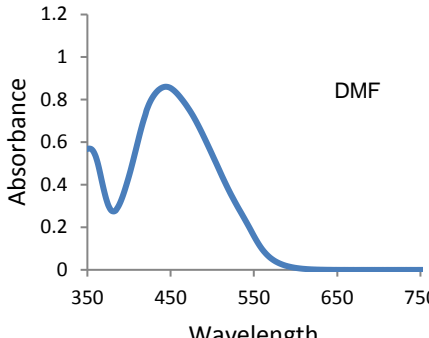
Although data were not requested for Procedure 2 (organic solvent–soluble colouring matters), the Committee noted that chloroform is listed as a reagent in that procedure. The Committee was reminded of previous efforts to remove this reagent from test procedures and decided that efforts should be made to replace it.

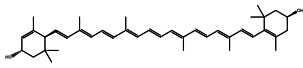
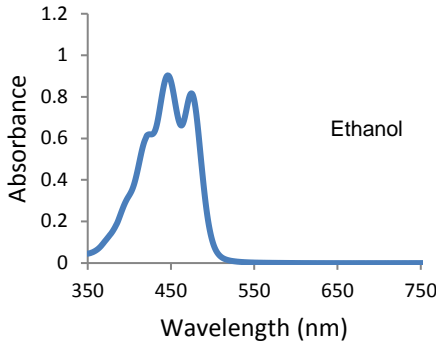
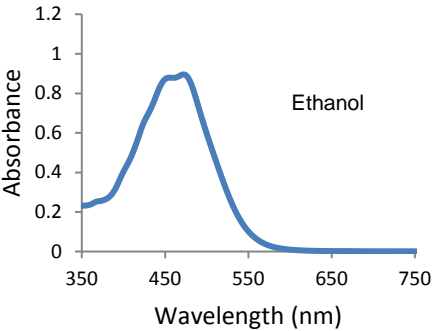
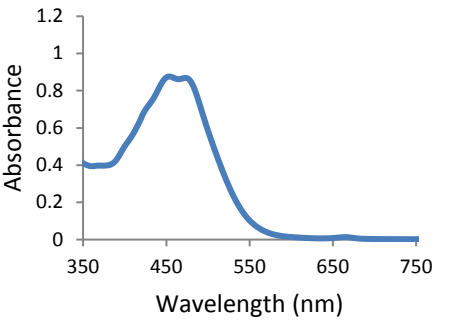
TOTAL COLOURING MATTERS CONTENT (VOLUME 4)

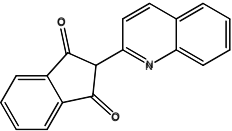
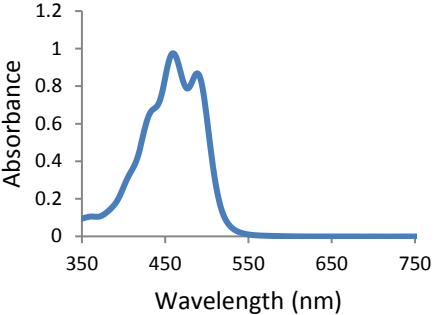
Visible Spectra: Replacement of Chloroform as Solvent

A = absorbance; Spec abs = specific absorbance ($\Delta_{1\text{ cm}}^{1\%}$); a = absorptivity (L/(g·cm))

JECFA NAME	STRUCTURE	SPECTRAL DATA	VISIBLE ABSORPTION SPECTRUM
Annatto Extracts (Bixin) $C_{25}H_{30}O_4$ m. wt. 394.50 CAS No. 6983-79-5 C.I. 75120		Ethanol $\lambda_{\text{max}} = 459 \text{ nm}$ $A = 0.6932$ Spec abs = 2333 $a = 233.3$ THF/acetone (JECFA) $\lambda_{\text{max}} = 487 \text{ nm}$ Spec abs = 3090 $a = 309.0$	
B-Apo-Carotenal (Apocarotenal) $C_{30}H_{40}O$ m. wt. 416.64 CAS No. 1107-26-2 C.I. 40825		Ethanol $\lambda_{\text{max}} = 463 \text{ nm}$ $A = 0.9058$ Spec abs = 2263 $a = 226.3$ Chloroform (JECFA) $\lambda_{\text{max}} = 461 \text{ nm}$ Spec abs = 2640 $a = 264.0$	

JECFA NAME	STRUCTURE	SPECTRAL DATA	VISIBLE ABSORPTION SPECTRUM
<p>B-Carotene</p>		<p>Hexane $\lambda_{\text{max}} = 451 \text{ nm}$ $A = 1.0011$ Spec abs = 2570 $a = 257.0$ (avg of 2)</p> <p>Chloroform (JECFA) $\lambda_{\text{max}} = 455 \text{ nm}$ Spec abs = 2500 $a = 250.0$</p>	
<p>Chlorophyll b from spinach</p> <p>$\text{C}_{55}\text{H}_{70}\text{MgN}_4$ O_6 m. wt. 904.47 CAS No. 519-62-0 C.I. 75810</p>		<p>Ethanol $\lambda_{\text{max}} = 464, 649 \text{ nm}$ $A = 0.8495, 0.3191$ Spec abs = 849 at 464 nm $a = 84.9$ at 464 nm</p> <p>Diethyl ether (JECFA) $\lambda_{\text{max}} = 660.4 \text{ nm}$ plus additional for simultaneous equations</p>	
<p>Curcumin (Synthetic)</p> <p>$\text{C}_{21}\text{H}_{20}\text{O}_6$ m. wt. 368.39 CAS No. 458-37-7 C.I. 75300</p>		<p>Ethanol $\lambda_{\text{max}} = 427 \text{ nm}$ $A = 1.0768$ Spec abs = 1606 $a = 160.6$ (avg of 2)</p> <p>Ethanol (JECFA) $\lambda_{\text{max}} = 425 \text{ nm}$ Spec abs = 1607 $a = 160.7$</p>	
<p>Lithol Rubin BK</p> <p>$\text{C}_{18}\text{H}_{12}\text{CaN}_2\text{O}_6\text{S}$ m. wt. 424.45 CAS No. 5284-04-9 C.I. 15850:1</p>		<p>Dimethylformamide $\lambda_{\text{max}} = 444 \text{ nm}$ $A = 0.8605$ Spec abs = 246 $a = 24.5$ (avg of 2) 94% total color</p> <p>Dimethylformamide (JECFA) $\lambda_{\text{max}} = 442 \text{ nm}$ Spec abs = not in monograph $a =$ not in monograph</p>	

JECFA NAME	STRUCTURE	SPECTRAL DATA	VISIBLE ABSORPTION SPECTRUM
Lutein $C_{40}H_{56}O_2$ m. wt. 568.87 CAS No. 127-40-2		Ethanol $\lambda_{max} = 446 \text{ nm}$ $A = 0.9031$ Spec abs = 1829 $a = 182.9$ (avg of 2) Chloroform/ethanol (JECFA) $\lambda_{max} = 445$ Spec abs = 2550 $a = 255.0$	
Paprika Extract $C_{40}H_{56}O_3$ m. wt. 584.89 CAS No. 465-42-9		Ethanol $\lambda_{max} = 472 \text{ nm}$ $A = 0.8955$ $\sim 13.6\%$ Acetone (JECFA) $\lambda_{max} = 462 \text{ nm}$ Spec abs = 2100 $a = 210.0$	
Paprika Oleoresin $C_{40}H_{56}O_3$ m. wt. 584.89 CAS No. 68917-78-2		Ethanol $\lambda_{max} = 473 \text{ nm}$ $A = 0.8685$ $\sim 23.4\%$ Acetone (JECFA) $\lambda_{max} = 462 \text{ nm}$ Spec abs = 2100 $a = 210.0$	

Impurity	Structure	Spectral data	Visible absorption spectrum
2-(2-quinolyl)- 1,3- indandione		Ethanol $\lambda_{max} = 414 \text{ nm}$ $A = 0.9415$ Spec abs = 1287 $a = 128.7$ Ethyl acetate (FDA) $\lambda_{max} = 418 \text{ nm}$ Spec abs = 1340 $a = 134.0$	

**The following monograph was erroneously omitted in the JECFA
Monograph 19**

ERRATA: CAROB BEAN GUM

Prepared at the 82nd JECFA and published in FAO JECFA Monographs 19 (2016) superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 25th JECFA (1981).

SYNONYMS

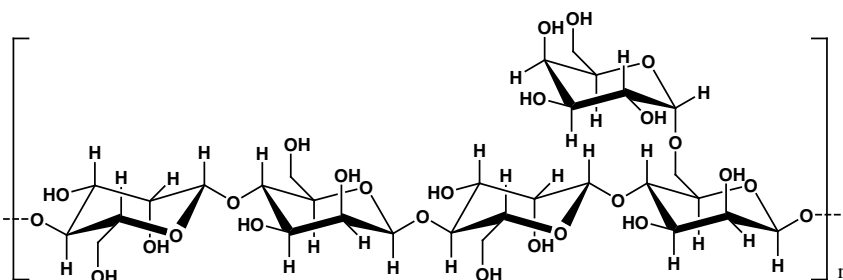
Locust bean gum, INS No. 410

DEFINITION

Carob bean gum, also known as locust bean gum, is a galactomannan polysaccharide obtained from the seeds of *Ceratonia siliqua* (L.) Taub. (Fam. Leguminosae). The ground endosperm of the seeds consists mainly of high molecular weight (approximately 50,000-3,000,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 4:1. The seeds are dehusked by treating the seeds with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ followed by milling and screening of the endosperm to obtain native carob bean gum. The gum may be washed with ethanol or isopropanol to control the microbiological load (washed carob bean gum).

C.A.S. number

9000-40-2

Structural formula**DESCRIPTION**

White to yellowish white, nearly odourless powder

FUNCTIONAL USES

Thickener, stabilizer, emulsifier, gelling agent

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Insoluble in ethanol
<u>Gel formation</u>	Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.
<u>Viscosity</u>	Transfer 2 g of the sample into a 400-mL beaker and moisten thoroughly with about 4 mL of isopropanol. Add 200 mL of water with vigorous stirring until the gum is completely and uniformly dispersed. An opalescent, slightly viscous solution is formed. Transfer 100 mL of this solution into another 400-mL beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).
<u>Gum constituents</u> (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.
<u>Microscopic examination</u>	Disperse a sample of the gum in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. Carob bean gum contains long stretched tubiform cells, separated or slightly interspaced. Their brown contents are much less regularly formed than in Guar gum.
PURITY	
<u>Loss on drying</u> (Vol. 4)	Not more than 14% (105°, 5 h)
<u>Total ash</u> (Vol. 4)	Not more than 1.2% (800°, 3-4 h)
<u>Acid-insoluble matter</u> (Vol. 4)	Not more than 4.0%
<u>Protein</u> (Vol. 4)	Not more than 7.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under "General Methods, Inorganic components"). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.
<u>Starch</u>	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced
<u>Residual solvents</u>	Not more than 1% of ethanol or isopropanol, singly or in combination See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg

Not more than 0.5 mg/kg for use in infant formula and in formulae for special medical purposes intended for infants.

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 methods described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria
(Vol. 4)

Initially prepare a 10⁻¹ dilution by adding a 50 g sample to 450 mL of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender.

Total (aerobic) plate count: Not more than 5,000 CFU/g

E. coli: Negative in 1g

Salmonella: Negative in 25 g

Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Method I)

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 to within 0.01 mg.

Standard solution: Add 50.0 mL water to a 50 mL vial and seal. Accurately weigh and inject 15 µl ethanol and weigh to within 0.01mg. Inject 15 µl isopropanol through the septum 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: Accurately weigh 0.500±0.001 g of sample in a small weighing boat. Pipette 5 mL of water and 1 mL internal standard solution into a headspace vial. 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.

CORRIGENDUM

The following requests for corrections, reported to the JECFA secretariats, were evaluated by the 84th 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 explanations
Carob bean gum (clarified) (JECFA 82, FAO JECFA Monographs 19, 2-16)	Heading: Carob Bean gum	Heading: Carob bean gum(clarified)	In the original publication of FAO JECF monograph 19, the monograph heading omitted (“(clarified)”), while the specifications referred to the clarified carob bean gum
Carob bean gum (JECFA 82, FAO JECFA Monographs 19, 2016)	None Specifications had been prepared and adopted at the 82 nd JECFA for carob bean gum but were not published in the JECFA Monograph 19.	(full monograph for Carob bean gum is reprinted above as errata in this Monograph)	
CITREM (JECFA 82, FAO JECFA Monographs 19, 2016)	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).	Lead (Vol. 4) Not more than 2 mg/kg. (Not more than 0.5 mg/kg for use in infant formula and formula for special medical purposes intended for infants). <i>[emphasis added for clarity only]</i>	Transcription error
Diammonium hydrogen phosphate (JECFA 59, FAO JECFA Monographs 1, 2006)	CAS 7783-54-0	CAS 7783-28-0	
Dimethyl Dicarbonate (JECFA 63, FAO JECFA Monographs 1, 2006)	CAS 004-525-33-1	CAS 4525-33-1	
Ferrous Sulfate (JECFA 53, FAO JECFA Monographs 1, 2006)	CAS 7720-78-7	CAS 7782-63-0	

Food additive	Original text	New text	Additional explanations
Ferrous Sulfate, Dried (JECFA 53, FAO JECFA Monographs 1, 2006)	No CAS number	CAS 7720-78-7	
Paprika Extract (JECFA 79, FAO JECFA Monographs 16, 2014)	Preamble: An ADI of 0 - 1.5 mg/kg bw was allocated at the 79th JECFA (2014).	Preamble: An ADI of 0 - 1.5 mg/kg bw (expressed as total carotenoids) was allocated at the 79 th JECFA (2014). <i>[emphasis added for clarity only]</i>	
Paprika Oleoresin (JECFA 59, FAO JECFA Monographs 1, 2006)	INS 160c	INS160c(i)	
L-Malic Acid (flavouring)	Optical rotation: - 0.23 (25°)	Optical rotation: - 2.3 (8.5 g/100 mL water at 20°)	Information was incomplete and erroneous. The optical rotation depends in size and orientation on solvent and concentration o L-Malic acid

ANNEX I: SUMMARY OF RECOMMENDATIONS FROM THE 84th JECFA

FOOD ADDITIVES CONSIDERED FOR SPECIFICATIONS ONLY

Food additive	Specifications
Microcrystalline cellulose	<u>R^a</u>
Silicon dioxide, amorphous	<u>R^b</u>
Sodium aluminium silicate	<u>R^c</u>
Steviol glycosides	<u>R^d</u>
Sucrose esters of fatty acids	<u>R^e</u>

R: existing specifications revised

^a The Committee assessed the information submitted on the solubility of microcrystalline cellulose and redesignated its solubility as “Insoluble in water and ethanol. Practically insoluble or insoluble in sodium hydroxide solution (50 g/L)”.

^b Silicon dioxide, amorphous was on the agenda at the present meeting for revisions related to pH, assay, loss on drying, loss on ignition and impurities. The Committee at its present meeting received the requested information. The tentative status was removed.

^c At the current meeting, the Committee evaluated the data submitted for loss on ignition, impurities soluble in 0.5 mol/L hydrochloric acid and the suitability of the proposed assay method for the determination of aluminium, silicon and sodium. Information received on functional uses confirmed that the substance is used only as an anticaking agent. The tentative status was removed.

^d The Committee received a validated HPLC–ultraviolet (UV) method for the assay of steviol glycosides, for which reference standards are commercially available. The presence of steviol glycosides in small quantities is confirmed using an HPLC–mass spectrometric method and quantified using HPLC–UV data. The Committee also received assay data for three batches of a commercial product using the proposed methods. The Committee, at its present meeting, assessed the information received and replaced the existing assay. Two additional saccharides (galactose and arabinose) have been identified in the extracts of *Stevia rebaudiana* Bertoni since the last evaluation of steviol glycosides. The Committee included the two saccharides in the definition of the specifications for steviol glycosides from *S. rebaudiana* Bertoni. The tentative status was removed.

The Committee received additional information pertaining to enzymatically modified steviol glycosides; however, the Committee noted that the data were outside the scope of the call for data for the current meeting and therefore did not consider them.

^e The Committee assessed the information submitted on the solubility of sucrose esters of fatty acids and revised the solubility criterion. In addition, the Committee reviewed the information submitted on the chromatographic conditions for the separation of the compounds and revised the UV integration instructions.

FOOD ADDITIVES EVALUATED TOXICOLOGICALLY AND ASSESSED FOR DIETARY EXPOSURE

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Brilliant Blue FCF	R ^a	<p>The Committee concluded that the available data support the revision of the ADI for Brilliant Blue FCF. In a long-term toxicity study in rats, a no-observed-adverse-effect level (NOAEL) of 631 mg/kg body weight (bw) per day was identified, based on a 15% decrease in mean terminal body weight and decreased survival of females at 1318 mg/kg bw per day. The Committee established an ADI of 0–6 mg/kg bw based on this NOAEL by applying an uncertainty factor of 100 for interspecies and intraspecies differences.</p> <p>The Committee noted that the conservative dietary exposure estimate of 5 mg/kg bw per day (95th percentile for children) is less than the upper limit of the ADI of 0–6 mg/kg bw established for Brilliant Blue FCF and concluded that dietary exposure to Brilliant Blue FCF for children and all other age groups does not present a health concern. The previous ADI of 0–12.5 mg/kg bw was withdrawn.</p>
β-Carotene-rich extract from <i>Dunaliella salina</i>	N	<p>The Committee noted that data have become available since the previous evaluation that show large differences in absorption of β-carotene between rodents and humans. The Committee considered that rodents are inappropriate animal models for establishing an ADI for β-carotene.</p> <p>The Committee noted that the toxicity of the other components of the β-carotene-rich d-limonene extract of <i>D. salina</i> (hereafter referred to as <i>D. salina</i> d-limonene extract) can be evaluated using the results of rodent studies. A short-term toxicity study in rats gave a NOAEL of 3180 mg/kg bw per day, the highest dose tested. No long-term toxicity or reproductive studies have been conducted. The <i>D. salina</i> d-limonene extract did not show genotoxicity or developmental toxicity. Correction of the NOAEL of 3180 mg/kg bw per day for the percentage of the algal component (20–35%) gives an adjusted NOAEL of 636–1113 mg/kg bw per day for the algal lipid component of the <i>D. salina</i> d-limonene extract. The margin of exposure for this algal lipid component is 2120–3710 using a dietary exposure of 18 mg/day (0.3 mg/kg bw per day). The Committee concluded that exposure to the algal component of the extract does not pose a health concern.</p> <p>The Committee noted that the total dietary exposure to β-carotene is not expected to increase when <i>D. salina</i> d-limonene extract is used as a food colour.</p> <p>The Committee concluded that there was no health concern for the use of β-carotene-rich extract from <i>D. salina</i> when used as a food colour in</p>

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Fast Green FCF	R ^a	<p>accordance with the specifications established at this meeting. The Committee emphasized that this conclusion applies to the use of this extract as a food colour, not as a food supplement.</p> <p>The ADI of 0–25 mg/kg bw established previously by the Committee was based on a long-term rat dietary that identified a NOAEL of 5% Fast Green FCF (equivalent to 2500 mg/kg bw per day), the highest concentration tested.</p> <p>The Committee concluded that the new data that had become available since the previous evaluation gave no reason to revise the ADI and confirmed the ADI of 0–25 mg/kg bw. The Committee noted that the conservative dietary exposure estimate for Fast Green FCF of 12 mg/kg bw per day (95th percentile for adolescents) was below the upper bound of the ADI. The Committee concluded that dietary exposures to Fast Green FCF for adolescents and all other age groups do not present a health concern.</p>
Gum ghatti	R ^b	<p>The Committee took into account the lack of systemic exposure to gum ghatti because of its high molecular weight and polysaccharide structure, its lack of toxicity in short-term studies, the lack of concern for genotoxicity and the absence of treatment-related adverse effects in studies of gum arabic and other polysaccharide gums with a similar profile.</p> <p>The Committee concluded that gum ghatti is unlikely to be of health concern and established an ADI “not specified”^c for gum ghatti that complies with the specifications.</p> <p>The Committee concluded that the estimated dietary exposure to gum ghatti of 12 mg/kg bw per day does not present a health concern.</p>
Jagua (Genipin–Glycine) Blue	N,T	<p>The Committee noted that the highest doses tested in two 90-day toxicity studies in rats and dogs were only 330 and 338 mg/kg bw per day (expressed on a “blue polymer” basis^d), respectively. The Committee was concerned that the possible effects of the low molecular weight component of the “blue polymer” that could be absorbed were not adequately investigated.</p> <p>A comparison of the dietary exposure estimate (11 mg/kg bw per day) with the NOAEL from the 90-day studies of oral toxicity in rats and dogs (approximately 330 mg/kg bw per day) gives a margin of exposure of about 30.</p> <p>Because of the limited biochemical and toxicological database and the low margin of exposure, the Committee was unable to complete the evaluation for Jagua (Genipin–Glycine) Blue.</p>
Metatartaric acid	T	<p>As metatartaric acid undergoes enzymatic hydrolysis to tartaric acid prior to systemic absorption, the</p>

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Tamarind seed polysaccharide	N	<p>biochemical and toxicological data on tartaric acid considered at previous meetings are relevant to the safety assessment of metatartaric acid. Previously evaluated and new studies suggest no change to the group ADI previously established for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.</p> <p>The Committee concluded that metatartaric acid (when used in winemaking) should be included in the group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium, potassium–sodium salts, expressed as L(+)-tartaric acid.</p> <p>The Committee noted that the dietary exposure estimate for metatartaric acid for adult consumers of wine was 4% of the upper bound of the ADI and concluded that dietary exposure to metatartaric acid in wine at the maximum use level of 100 mg/L does not present a health concern.</p>
Tannins (oenological tannins)	–	<p>The Committee noted the absence of toxicity in long-term rodent studies and lack of concern regarding genotoxicity, reproductive toxicity and developmental toxicity, and established an ADI “not specified”^c for tamarind seed polysaccharide.</p> <p>The Committee concluded that the estimated dietary exposure of 75 mg/kg bw per day based on proposed uses and use levels does not present a health concern.</p> <p>The Committee noted that the available data do not provide clear information on which tannin sources and individual tannin compounds are present in commercially used oenological tannins and, thus, how the oenological tannins would compare to the tannins used in the submitted studies. Therefore, it is not possible to establish which studies are relevant and, consequently, the extent of the data gaps.</p> <p>The information on biochemical aspects is incomplete, with the implications of repeated dosing on absorption, tissue distribution and interindividual variation needing consideration. In general, there are also few data available on reproductive and developmental toxicity and/or long-term toxicity for some or all of the tannins.</p> <p>In the absence of specifications and identification of the products in commerce, the Committee concluded that it was not possible to evaluate tannins used in winemaking.</p>
Yeast extracts containing mannoproteins	N,T	<p>In addition to the natural presence of yeast mannoproteins in wine and the long history of consumption of yeast products in common foods, the Committee considered that the tentative product specifications for yeast extracts containing mannoproteins indicate that these do not contain chemical residues or microbiological contaminants of</p>

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		<p>concern. In addition, the Committee estimated that dietary exposure to yeast mannoproteins due to the addition of yeast extracts containing mannoproteins to wine at the maximum level of 400 mg/L would result, on average, in a 20% increase in dietary exposure compared to the background exposure through the regular diet of 0.4–21 mg/kg bw per day, primarily driven by bread and pastries. These conservative dietary exposure estimates are based on the assumption that 100% of the yeast extracts containing mannoproteins is mannoproteins.</p> <p>In considering the data and information regarding yeast and yeast-derived products, the Committee concluded that it is unlikely that there would be a health concern for the use of yeast extracts containing mannoproteins as a food additive for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.</p> <p>The Committee noted that any change in the uses and/or use levels of yeast extracts containing mannoproteins as a food additive will require a new evaluation.</p>

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

- ^a A maximum limit for manganese was added. High-performance liquid chromatography (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 or aqueous ammonium acetate.
- ^b An HPLC method for the identification of the gum constituents was added to replace the thin-layer chromatography (TLC) method. One identification method, using a mercury-containing reagent, was removed. L-Rhamnose was added as one of the constituents of gum ghatti, based on current literature reports.
- ^c ADI “not specified” is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect; it should not conceal food of inferior quality or adulterated food; and it should not create a nutritional imbalance.
- ^d “Blue polymer” refers to the blue-coloured genipin–glycine polymer and dimer content of Jagua (Genipin–Glycine) Blue.

ANNEX 2. GENERAL INFORMATION

INFORMATION REQUIREMENTS FOR SUBMISSIONS ON PRODUCTS DERIVED FROM NATURAL SOURCES

The Committee noted that, at the current meeting, a number of food additives were evaluated that were derived from natural sources. The Committee recalled that at previous meetings, the need for sponsors to provide sufficient data for chemical, technical, dietary exposure and toxicological evaluation was stressed. At its thirty-first meeting, the Committee emphasized that “A full understanding of the source and chemical nature of such products was considered essential for an evaluation of their safety-in-use”. At the sixty-eighth meeting, the Committee provided considerations on “Extensions of an existing ADI to substances obtained from different sources and/or by different manufacturing processes”.

The Committee recognized that a component of interest (e.g. carotenes) may be present in the product of commerce at a low percentage relative to other components either because it is extracted together with components of similar polarity or solubility or because of subsequent standardization in the final product formulation. The Committee also recognized that some substances (e.g. gums or tannins) are complex mixtures and their components are affected to varying degrees, depending on their source or through processing. It is important to fully characterize all components of the final product, taking care to also provide the detailed manufacturing process as well as information on the carryover of substances from the starting material to the final product.

The present Committee again stressed that a full characterization of the products in commerce and a relevant set of biochemical and toxicological data on such products are essential for the Committee to develop a specifications monograph and the related safety assessment. It is not possible to complete the evaluation of a food additive if its composition cannot be compared to the substances tested biochemically and toxicologically. This is particularly important where the submission relies on literature data.

The Committee encourages the Codex Committee on Food Additives (CCFA) to consider the above information requirements before accepting proposals for food additive evaluations to be included in the CCFA priority list.

ANNEX 3. FUTURE WORK AND RECOMMENDATIONS

β -Carotene-rich extract from *Dunaliella salina*

The Committee considered the basis for the ADI established for the group of carotenoids by the Committee at the eighteenth meeting. The group ADI (0–5 mg/kg bw) was derived using a four-generation study in rats with a NOAEL for β -carotene of 50 mg/kg bw per day with application of a safety factor of 10 because of the natural occurrence of carotenoids in the human diet and the low toxicity observed in animal studies. This ADI applies to the use of β -carotene as a colouring agent and not to its use as a food supplement.

Data that have become available since the previous evaluation show large differences in absorption of β -carotene between rodent species and humans. Specific β -carotene-15,15'-dioxygenase activity with β -carotene as substrate in the intestine of rodents is nearly 1 million-fold higher than that of humans. The Committee considered that rodents are inappropriate animal models for establishing an ADI for β -carotene because of the virtual absence of systemic absorption in rodents.

The Committee recommends that the group ADI for the sum of carotenoids, including β -carotene, β -apo-8'-carotenal and β -apo-8'-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β -carotene in rodents and rabbits in contrast to humans.

Jagua (Genipin–Glycine) Blue

The Committee raised concern regarding the potential toxicity of a low molecular weight fraction of the total colouring matter in Jagua (Genipin–Glycine) Blue. The Committee recommends additional biochemical and toxicological information (e.g. absorption, distribution, metabolism and excretion studies and long-term toxicity, carcinogenicity, reproductive and developmental toxicity studies), including the use of higher doses of the “blue polymer” (which refers to the blue-coloured genipin–glycine content of Jagua (Genipin–Glycine) Blue), including the dimers, in order to complete an evaluation of the safety of Jagua (Genipin–Glycine) Blue.

To support the above, additional information is required on:

- characterization of the low molecular weight components of the “blue polymer”;
- a validated method for the determination of dimers; and
- data on concentrations of dimers from five batches of the commercial product.

Metatartaric acid

The Committee received limited analytical data on metatartaric acid. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested:

- characterization of the products (optical rotation, content of free tartaric acid, degree of esterification and molecular weight distribution) and the corresponding analytical methods;
- infrared spectrum (in a suitable medium); and
- analytical results including the above parameters from a minimum of five batches of products currently available in commerce, along with quality control data.

The Committee requests that this information be submitted by **December 2018**.

Tannins

The Committee assessed the information received and concluded that there were insufficient data and information to prepare specifications for oenological tannins. The Committee requires data for the characterization of the products in commerce to be able to complete specifications for oenological tannins used as an antioxidant, colour retention agent and stabilizer in wine. The required information includes a detailed description of the manufacturing processes and thorough chemical characterization of the commercial products made from different botanical sources.

The following information is required:

- composition of tannins derived from the full range of raw materials as well as the processes used in their manufacture;
- validated analytical method(s) and relevant quality control data;
- analytical data from five batches of each commercial product including information related to impurities such as gums, resinous substances, residual solvents, sulfur dioxide content and metallic impurities (arsenic, lead, iron, cadmium and mercury);
- solubility of the products in commerce, according to JECFA terminology; and
- use levels, natural occurrence and food products in which tannins are used.

Submitters are encouraged to offer a rationale for a single specifications monograph for oenological tannins covering all products or individual monographs.

Yeast extracts containing mannoproteins

In order to complete specifications related to the use of yeast extracts containing mannoproteins in wine manufacture and remove their tentative designation, the Committee requires chemical characterization of the product in commerce along with supporting data. The following information is required:

- composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;
- analytical data from five batches of each commercial product, including information related to impurities; and
- data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.

The Committee requests that this information be submitted by **December 2018**.

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives
84th Meeting 2017

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 Rome, 6–15 June 2017. 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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