SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for the following food additives and these are provided in this publication:

Activated carbon (R)
Cassia gum (R)
Indigotine (R)
Steviol glycosides (R)
Sucrose esters of fatty acids (R)
Sucrose monoesters of lauric, palmitic or stearic acid (N, T)
Titanium dioxide (R)

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

New and revised INS numbers assigned to food additives by the Codex Alimentarius Commission at its 33rd session in 2010, (ALINORM 10/33/12, Appendix IX) and a correction for the INS number for Stannous chloride to No. 512, have been introduced in the corresponding JECFA food additive specifications monographs in the on-line database, as appropriate, and these are not reproduced in this publication.

Minor editorial revisions and corrections to the limits and information relating to metals and arsenic as published in FAO JECFA Monographs 1 (2005, 2006), Combined Compendium of Food Additive Specifications, have been made to the following JECFA food additive specifications monographs in the on-line database and are not reproduced in this publication: Carotenes (Algae), Carotenes (Vegetable), Calcium silicate, Ferric ammonium citrate, Grape skin extract, Potassium carbonate, Trimagnesium phosphate and Trisodium phosphate. The corrected limits correspond to those agreed by the Committee and published in the reports of JECFA from the relevant meetings (57th, 59th and 63rd meetings of JECFA).

ACTIVATED CARBON

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 37th JECFA (1990) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). No ADI was established at the 31st JECFA (1987).

SYNONYMS Activated charcoal, decolourizing carbon

DEFINITION A solid, porous, carbonaceous material prepared by carbonizing and activating organic substances. The raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, petroleum coke, etc., may be carbonized and activated at high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide. Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride and the mixture carbonized at an elevated temperature, followed by removal of the chemical activating agent by water washing.

Chemical names Carbon

C.A.S. number 7440-44-0

Chemical formula C

Formula weight 12.01

DESCRIPTION Powder or granules, black, odourless

FUNCTIONAL USES Adsorbent, decolourizing agent

GENERAL SPECIFICATIONS Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

CHARACTERISTICS

IDENTIFICATION

Solubility Adsorbent, decolourizing agent

Adsorption Place about 3 g of powdered sample in a glass-stoppered flask containing 10 ml of dilute hydrochloric acid (5%), boil for 30 s, and cool to room temperature. Add 100 ml of iodine TS, stopper, and shake vigorously for 30 sec. Filter through filter paper (Whatman No. 2 or equivalent), discarding the first portion of filtrate. Compare 50 ml of the filtrate with a reference solution prepared by diluting 10 ml of iodine to 50 ml with water, but not treated with carbon. The colour of the carbon treated iodine solution shall be lighter in colour than that of the reference solution, indicating the adsorptivity of the sample.

PURITY

Adsorption power	Not less than 90% and not more than 110% of the value stated on label. See description under TESTS
Loss on drying (Vol. 4)	Not more than 15% (120º, 4 h) (See Volume 4 under "GENERAL METHODS, Inorganic Components.")
Sulfide compounds	To 1.0 g of the sample in a conical flask add 5 ml of 1 N hydrochloric acid and 20 ml of water. Heat to boiling. The fumes released do not turn lead acetate paper brown. (Lead acetate paper is prepared by saturating filter paper with lead acetate TS and drying the paper at 100º).
Acid soluble substances	Not more than 3% To about 1 g of the sample, accurately weighed, add 25 ml of dilute nitric acid TS and boil for 5 min. Filter whilst hot through a sintered-glass filter (10) and wash with 10 ml of hot water. Evaporate the combined filtrate and washings to dryness on a water bath, add to the residue 1 ml of hydrochloric acid, evaporate to dryness again and dry the residue to constant weight at 103±2º.
Sulfated ash	Not more than 5% Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator and weigh. Accurately weigh about 1 g of sample in the crucible and add 2 ml of sulfuric acid TS. Heat at first on a water bath, then cautiously over a flame, then progressively to about 600º. Continue the incineration until all black particles have disappeared and allow the crucible to cool. Add a few drops of dilute sulfuric acid TS, heat and incinerate as before and allow to cool. Evaporate and incinerate carefully, allow to cool, weigh, and repeat the ignition for 15 min to constant weight.
Water extractable substances	Not more than 4% Transfer about 5 g of sample, accurately weighed, into a 250 ml flask provided with a reflux condenser and a Bunsen valve. Add 100 ml of water and several glass beads, and reflux for 1 h. Cool slightly, and filter through Whatman No 2 or equivalent filter paper, discarding the first 10 ml of filtrate. Cool the filtrate to room temperature, and pipet 25.0 ml into a tared dish. Evaporate the filtrate in the dish to incipient dryness on a hot plate never allowing the solution to boil. Dry for 1 h at 103±2º in a vacuum oven, cool and weigh. Calculate the percentage of water extractables in the filtrate, based on the sample weight and volume of sample taken for gravimetric measurement.
Alcohol soluble substances	Not more than 0.5% To 2.0 g of sample add 50 ml of ethanol (96 per cent) and boil under a reflux condenser for 10 min. Filter immediately, wash residue with 10 ml of warm ethanol and filter. Quantitatively transfer the combined filtrate into a tared beaker containing a few antibumping stones. Evaporate to dryness on a water bath and dry to a constant mass at 103±2º. The residue on evaporation weighs not more than 10 mg**.**
Alkali soluble coloured substances	To 0.25 g of sample add 10 ml of 2 N sodium hydroxide and boil for 1 min. Cool, filter and dilute the filtrate to 10 ml with water. Prepare a

reference solution by mixing 1.90 ml of solution A (1% hydrochloric acid) and 0.10 ml of a solution B (9.6 ml of ferric chloride TS + 0.2 ml of cobaltous chloride TS + 0.2 ml of cupric sulfate TS). The colour of sample solution shall not be more intense than that of the reference solution.

Cyanogen compounds — Mix 5 g of sample with 50 ml of water and 2 g of tartaric acid. Distil the mixture, collecting 25 ml of distillate below the surface of a mixture of 2 ml of sodium hydroxide TS and 10 ml of water contained in a small flask placed in an ice bath. Dilute the distillate to 50 ml with water, and mix. Add 12 drops of ferrous sulfate TS to 25 ml of the diluted distillate, heat almost to boiling, cool, and add 1 ml of hydrochloric acid. No blue colour is produced.

Higher aromatic hydrocarbons — Extract 5 g of the sample with about 45 ml of cyclohexane in a continuous extraction apparatus for 2 h. Collect the extract and dilute to 50 ml with cyclohexane. Examine under ultraviolet light at 365 nm. The colour or fluorescence of the solution is not more intense than that of a 83 ng/ml solution of quinine prepared in 0.01N sulfuric acid, examined under the same conditions.

Arsenic (Vol. 4) — Not more than 3 mg/kg
Accurately weigh about 4 g of the sample into a conical flask, add 80 ml of 2 N hydrochloric acid, extra pure, and boil gently under reflux for 1 h, filter and wash the filter with 2 N hydrochloric acid. Cool and quantitatively transfer the filtrate into 100 ml volumetric flask and make up to volume with the same acid. Determine arsenic using atomic absorption hydride generation technique.

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level using the solution prepared under arsenic.

Zinc (Vol.4) — Not more than 25 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level using the solution prepared under arsenic.

TESTS

PURITY TESTS

Adsorption power — To about 0.3 g of dried sample, accurately weighed, in a 100 ml ground-glass-stoppered conical flask, add 25.0 ml of a freshly prepared solution of 0.5 g of phenazone in 50 ml of water. Shake thoroughly for 15 min. Filter and reject the first 5 ml of filtrate. Pipette 10.0 ml of the filtrate into a conical flask, add 1.0 g of potassium bromide and 20 ml of dilute hydrochloric acid TS. Using 0.1 ml of ethoxychrysoidine solution as indicator, titrate with 0.1 N potassium bromate until the colour changes from reddish-pink to yellowish-pink. Titrate slowly (1 drop every 15 sec) towards the end of the titration. Carry out a blank titration using 10.0 ml of the phenazone solution.

Calculate adsorption power from:

$$[235.3 (a - b)]/[d \times m]$$

where

a is the volume (ml) of 0.1 N potassium bromate consumed by the blank;

b is the volume (ml) of 0.1 N potassium bromate consumed by the test solution;

m is the mass (g) of dried sample; and

d is the value stated on the label.

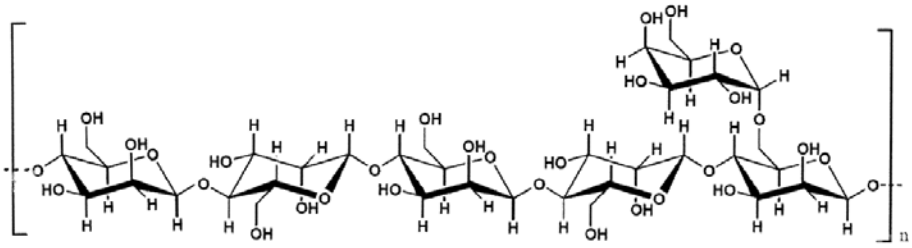
CASSIA GUM

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding tentative specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). An ADI "not specified" was established at the 71st JECFA (2009).

SYNONYMS INS 427

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

Structural formula



Assay Not less than 75% of galactomannan

DESCRIPTION Pale yellow to off-white, odourless free-flowing powder

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

CHARACTERISTICS

IDENTIFICATION

Solubility
Insoluble in ethanol
Disperses well in cold water forming colloidal solutions.

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

Gel formation with xanthan gum
Passes test
See description under tests

Gum constituents (Vol. 4)
Proceed as directed under Gum Constituents Identification (Vol. 4) using 100 mg of sample instead of 200 mg and 1-10 µl of the hydrolysate instead of 1-5 µl. Use galactose and mannose as reference standards. These constituents should be present.

Viscosity	Less than 500 mPas (25º, 2h) (1% solution) See description under TESTS
pH (Vol. 4)	5.5-8.0 (1%)
PURITY	
Loss on drying (Vol. 4)	Not more than 12% (105º , 5 h)
Total ash (Vol. 4)	Not more than 1.2%
Acid-insoluble matter (Vol. 4)	Not more than 2.0%
Protein (Vol. 4)	Not more than 7.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method; Vol. 4). The percent of nitrogen in the sample multiplied by 6.25 gives the percent of protein in the sample.
Crude fat	Not more than 1% See description under TESTS
Starch	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
Anthraquinones	Not more than 0.5 mg/kg See description under TESTS
Residual solvents	Isopropanol: Not more than 1.0% See description under TESTS
Lead (Vol. 4)	Not more than 1 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 method described in Volume 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 cfu/g Yeast and mould: Not more than 100 cfu/g *E. coli*: Negative in 1 g *Salmonella*: Negative in 25 g

TESTS

IDENTIFICATION TESTS

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

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

Viscosity

Weigh 5 g of the sample in a plastic dish and 495 g of distilled water at 20º in a 1000 ml beaker. Add a magnetic bar and place the beaker on the agitation plate. Adjust the speed of agitation to 750 rpm. Introduce quickly the 5 g of sample in the water and cover the beaker with a watch glass. Keep the temperature at 90º for 15 min. Cool the solution at 25º (the cooling must be ±1.5º) in a water bath and measure the viscosity after 2 h at 25º using a RVT Brookfield Spindle 1, speed 20 rpm. Repeat the procedure with a sample of 5 g of carob (locust) bean gum.

(Note: The viscosity of the cassia gum (150 - 500 mPas) must be less than 50% that of carob bean gum (2000 - 3500 mPas))

PURITY TESTS

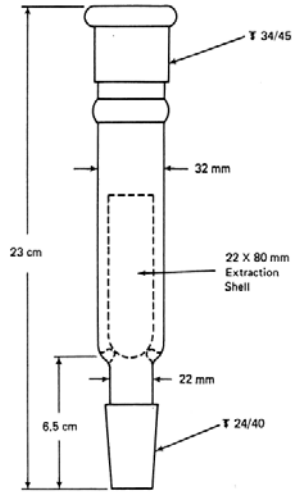
Crude fat

Apparatus

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

Procedure

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



Butt-Type Extractor for fat determination.

NOTE: The method for crude fat is referenced from the Food Chemicals Codex, 6th Edition, 2008, p. 1163.
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Anthraquinones

Principle

The antraquinones are extracted with acetonitrile and determined by High Performance Liquid Chromatography (Vol. 4) using the conditions below.
NOTE: Samples and standards should be protected from light.

Standards

Emodin (EMO), Aloe-emodin (AEM), Physcion (PHY) or 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone, Rhein (RHE) and Chrysophanic acid (CHR).
Internal standard: Danthrone (DAN) or 1,8-dihydroxy anthroquinone.

Use HPLC grade methanol for the solutions.

Stock standard solutions (100 mg/l): For each of the specific anthraquinone standards and for the internal standard: accurately weigh about 1 mg (±0.01 mg) of the standard. Transfer to 10 ml volumetric flasks with about 5 ml of methanol, sonicate for 15 min and dilute to volume with methanol.
Store these solutions in amber coloured bottles at 4° (the solutions are stable for 2 weeks under these conditions).

Mixed standard solution (10 mg/l):
Pipette 1 ml of each of the specific anthraquinone stock standard solutions into a 10 ml volumetric flask and dilute to volume with methanol.

Working standard solutions: To each of five 10 ml volumetric flask pipette 5, 2, 1, 0.5 and 0 ml respectively of the Mixed standard solution, pipette 1 ml of the Internal standard stock solution to each flask, mix and dilute to volume with methanol.

Sample preparation

Accurately weigh about 0.40 g of the sample into a 50 ml round-bottom flask. Add 20 ml trifluoroacetic acid and reflux at 70º for 4 hours. Cool the sample to ambient temperature and evaporate to dryness using a rotary evaporator. Add 3 ml of acetonitrile/$NaHCO_3$ (0.2%) (60:40 v/v) and sonicate for 30 min. Transfer the solution in a centrifuge tube and run it at 5000 rpm for 30 min. Filter the supernatant solution through an Extrulet column (Merck, NT1 or equivalent) previously neutralized with a pH 9.0 buffer. Pipette 900 µl of this filtered sample solution into a 2.5 ml volume vial and add 100 µl of the Internal standard stock solution and mix thoroughly.

Chromatographic conditions
Column: Hypersil C18 (250 mm x 4.6 mm ID, 5 µm) or equivalent
Mobile phase:
(A): 0.1% trifluoroacetic acid in water
(B): Acetonitrile (HPLC grade)
Injection volume: 50 µl
Run Time: 60 min
Gradient:

Min	% (A)	% (B)
0	86	14
10	86	14
15	80	20
25	80	20
55	20	80
60	0	100

Flow rate: 1 ml/min
Detector: Photodiode Array Detector. Quantification is performed at 435 nm

Standard curves
Inject 50 µl of each working standard solution and internal standard solution. Construct the standard curves by plotting the ratios of the peak areas of each of the specific anthraquinone / internal standard against the concentrations of each working standard solution (mg/l).

Procedure
Inject 50 µl of the Sample solution and the internal standard solution. Calculate the ratios of the peak areas of each specific anthraquinone / internal standard, and obtain the concentration (C) of each specific anthraquinone from the standard curves.

Calculate the percentage of each specific anthraquinone from:

$$\text{Anthraquinone (mg/kg)} = C \times 3 \times 1000 / (100 \times 0.9 \times W)$$

where
C is the concentration of specific anthraquinone (mg/l); and
W is weight of sample (g).

METHOD OF ASSAY The difference between 100 and the sum of the percent Loss on Drying, Total Ash, Acid-Insoluble Matter, Protein and Crude Fat represents the percent *Galactomannans*.

INDIGOTINE

Prepared at the 73rd JECFA (2010) and published in FAO Monographs 10 (2010), superseding specifications prepared at the 28th JECFA (1984) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0 - 5 mg/kg bw was established at the 18th JECFA (1974).

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

DEFINITION Consists essentially of a mixture of disodium 3,3' -dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate (principal component) and disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate (isomer) and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

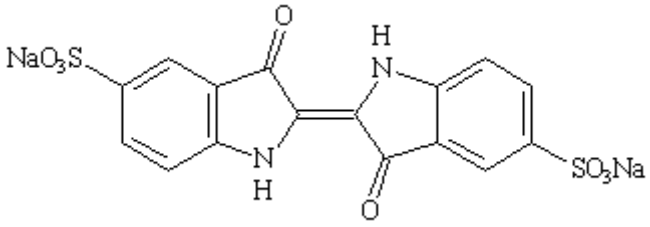
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names Disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate (principal component)

C.A.S. number 860-22-0 (principal component)

Chemical formula $C_{16}H_8N_2Na_2O_8S_2$ (principal component)

Structural formula



Principal component

Formula weight 466.36 (principal component)

Assay Not less than 85% total colouring matters.
Not more than 18% of disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate.

DESCRIPTION Blue powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Identification of colouring matters (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 15% at 135° together with chloride and sulfate calculated as sodium salts. (See Volume 4 under “SPECIFIC METHODS, Food Colours.”)
Water insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 1% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-sulfoanthranilic acid and anthranilic acid. (See Volume 4 under “SPECIFIC METHODS, Food Colours.”) Proceed as directed under *Determination by High Performance Liquid Chromatography* using an elution gradient of 2 to 100% at 4% per min (linear) followed by elution at 100%.
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline (See Volume 4 under “SPECIFIC METHODS, Food Colours.”)
Ether extractable matter (Vol. 4)	Not more than 0.2% (See Volume 4 under “SPECIFIC METHODS, Food Colours, Method II.”) Use 2 g of sample for the test.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Inorganic Components, Metallic Impurities”).

TESTS

PURITY TESTS

Subsidiary colouring matters	Subsidiary colouring matters are determined by high performance liquid chromatography using the following conditions: Chromatographic system - HPLC system with a UV/VIS detector or a diode array detector, auto sampler or injector - Detector wavelength: 610 nm

- Column: C18 on silica gel (250 x 4.6 mm, 5 µm) ACE 5 C18 or equivalent
- Mobile phase: solvent A: 0.02 mol/l ammonium acetate and solvent B: acetonitrile: water (7:3 v/v)
- Gradient elution: A:B 92:8 v/v to A:B 70:30 v/v (0-15 min); to A:B 40:60 v/v (15-25 min); to A:B 92:8 v/v (25-30 min); A:B 92:8 v/v (30-35 min).
- Column temperature: 40°
- Flow rate: 1.0 ml/min
- Injection volume: 20 µl

The subsidiary colours are separated from the principal component and its isomer. The subsidiary colouring matter monosodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5 sulfonate elutes at approximately 21 min.

METHOD OF ASSAY **Total colouring matters content**

Proceed as directed under *Total Content by Titration with Titanous Chloride* in Volume 4*,* using the following (See "SPECIFIC METHODS, Food Colours"):
Weight of sample: 1.0-1.1 g
Buffer: 15 g sodium hydrogen tartrate
Weight (D) of colouring matters equivalent to
1.00 ml of 0.1 N $TiCl_3$: 23.32 mg.

Disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate,disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate (5,7' isomer) and subsidiary colouring matters by HPLC

The isomers get separated under the HPLC conditions detailed under the separation of subsidiary colouring matters, and the amounts present can be quantified using an external standard calibration.

Reagents
- Acetonitrile, HPLC grade
- Ammonium acetate, HPLC grade
- Reference standards of disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate and disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate

Standard stock solutions (1000 µg/ml): Weigh accurately 0.10 g (±0.1 mg) of each reference standard and transfer to a 100 ml volumetric flask and bring to volume with water.

Standard solutions: Prepare five solutions from the standard stock solutions in the concentration range of 1 to 20 µg/ml.

Sample solution: Weigh accurately 0.10 g (±0.1 mg) (w_2) of the sample and transfer to a 100 ml volumetric flask (v) and bring to volume with water (sample solution S_A).
Transfer 1.00 ml (v_{s1}) and 5.00 ml (v_{s5}) of the solution S_A to two 50 ml volumetric flasks (v_S), respectively, and bring to volume with water (sample solutions S_1 and S_5).

Procedure

Inject the five standard solutions for each isomer using the conditions detailed under TESTS (Subsidiary colouring matters by HPLC) and integrate peak areas at 6.5 min for the 5,5’ isomer (disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate) and 10.8 min for the 5,7’ isomer (disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate). Construct standard curve for each compound (Area vs. standard concentration, µg/ml).
Inject sample solutions S_A, S_1 and S_5. The peak area of the 5,7’ isomer for the sample solution should be in the linear range of the calibration graph, otherwise increase the volume v_{s5}. Sample solutions S_1, S_5 and S_A are to quantify disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate, disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate (5,7’ isomer) and subsidiary colouring matters, respectively.

Calculations
Calculate the concentrations (C, % (w/w)) of the two isomers and subsidiary colouring matters in the sample using the standard curves and the following formulas:

$$C_{5.5'\,\text{isomer}\,\%\,(w/w)} = \left(\frac{A_{5,5} - b_{5,5'}}{m_{5,5'}} \right) \times 10^{-6} \times \frac{v_S}{v_{s1}} \times v \times \frac{100}{w_S}$$

$$C_{5.7'\,\text{isomer}\,\%\,(w/w)} = \left(\frac{A_{5,7} - b_{5,7'}}{m_{5,7'}} \right) \times 10^{-6} \times \frac{v_S}{v_{s5}} \times v \times \frac{100}{w_S}$$

$$C_{\text{Subsidiary colouring matters}\,\%\,(w/w)} = \left(\frac{A_{sum} - b_{5,5'}}{m_{5,5'}} \right) \times 10^{-6} \times v \times \frac{100}{w_S}$$

where
$A_{5,5'}$ is the area of the peak of the 5,5’ isomer in the sample chromatogram (area units);
$A_{5,7'}$ is the area of the peak of the 5,7’ isomer in the sample chromatogram (area units);
A_{sum} is the sum of the areas of the peaks in the chromatogram (610 nm), except for the two isomers;
$b_{5,5'}$ and $b_{5,7'}$ are the linear coefficients of the calibration graphs for the 5,5’ isomer and 5,7’ isomer, respectively;
$m_{5,5'}$ and $m_{5,7'}$ are is the slope of the calibration graph (area units ml/µg) for the 5,5’ isomer and 5,7’ isomer, respectively;
w_s is the sample weight (g);
v is the volume of the sample solution s_A (ml);
v_{s1} is the volume of the sample solution s_1 (ml); and
v_{s5} is the volume of the sample solution s_5 (ml).

STEVIOL GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION 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.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

C.A.S. number

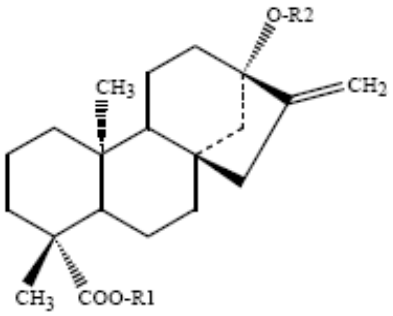
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The nine named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	*β*-Glc	*β*-Glc-*β*-Glc(2→1)
Rebaudioside A	*β*-Glc	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside B	H	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside C	*β*-Glc	*β*-Glc-*α*-Rha(2→1) | *β*-Glc(3→1)
Rebaudioside D	*β*-Glc-*β*-Glc(2→1)	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside F	*β*-Glc	*β*-Glc-*β*-Xyl(2→1) | *β*-Glc(3→1)
Dulcoside A	*β*-Glc	*β*-Glc-*α*-Rha(2→1)
Rubusoside	*β*-Glc	*β*-Glc
Steviolbioside	H	*β*-Glc-*β*-Glc(2→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay

Not less than 95% of the total of the nine named steviol glycosides on the dried basis.

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 water

Stevioside and rebaudioside A

The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or

rebaudioside A.

pH (Vol. 4) — Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4) — Not more than 1%

Loss on drying (Vol. 4) — Not more than 6% (105°, 2h)

Residual solvents (Vol. 4) — Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)

Arsenic (Vol. 4) — Not more than 1 mg/kg
Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)

Lead (Vol. 4) — Not more than 1 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 Vol. 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY — Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions.

Reagents
Acetonitrile: more than 95% transmittance at 210 nm.

Standards
Stevioside: more than 99.0% purity on the dried basis.
Rebaudioside A: more than 99.0% purity on the dried basis.
Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution
Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution
Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure
Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C_{18} MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter:

4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.
Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%\textit{Rebaudioside A} = [W_R/W] \times [A_X/A_R] \times 100$$

where
X is each steviol glycoside;
W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
W is the amount (mg) calculated on the dried basis of sample in the sample solution;
A_S is the peak area for stevioside from the standard solution;
A_R is the peak area for rebaudioside from the standard solution;
A_X is the peak area of *X* for the sample solution; and
f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

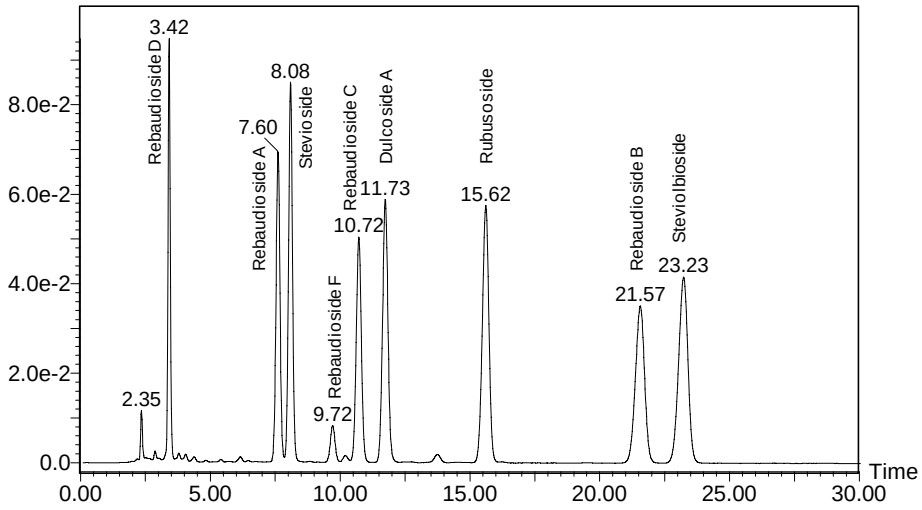


Figure. Chromatogram of mixture of nine steviol glycosides standard solution

Column: Capcell pak C_{18} MG II

Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

SUCROSE ESTERS OF FATTY ACIDS

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). 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) Sparingly soluble in water, soluble in ethanol

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 waterbath 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.

PURITY

Sulfated ash (Vol.4) Not more than 2%
Test 1 g of the sample (Method I)

Acid value (Vol.4) Not more than 6

Free sucrose Not more than 5%
See description under TESTS

Dimethylformamide	Not more than 1 mg/kg See description under TESTS
Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Ethyl acetate, isopropanol and propylene glycol	Not more than 350 mg/kg, singly or in combination See description under TESTS
Isobutanol	Not more than 10 mg/kg See description under TESTS
Methanol	Not more than 10 mg/kg See description under TESTS
Methyl ethyl ketone	Not more than 10 mg/kg See description under TESTS
Lead (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

Free sucrose

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
Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 280 °
- column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
- 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:
- injection port: 180°
- column: Hold for 2 min at 40°, then 40-160° at 20°/min, hold for 2 min at 160°
- 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 (C_{DFA}) from:

$$C_{DFA} \text{ (mg/kg)} = C \times 20 / W$$

where
C is dimethylformamide concentration determined (μg/ml); and
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
- injection port: 210°
- 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 (C_{DMSO}) from:

$$C_{DMSO} \text{ (mg/kg)} = C \times 25 / W$$

where
C is dimethyl sulfoxide concentration determined (μg/ml); and
W is weight of sample (g).

Propylene glycol

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

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

Standard solutions
Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 μg/ml of propylene glycol 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:
- injection port: 230°
- column: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
- 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 this centrifugal supernatant into the chromatograph.

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

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

C_{PG} (mg/kg) = C x 10 / W

where
C is polyethylene glycol concentration determined (μg/ml); and
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 1 g 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
Temperatures
- injection port: 110°
- column: 40°
- detector: 110°
Head space sampler:
- sample heat insulating temperature: 80°
- sample heat insulating period: 40 min
- syringe temperature: 85°
- 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 (mg/kg)= w_i / W

where
w_i is x-intercept of relationship line using the standard addition method (μg); and
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:
- Column: 40°
- 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; and
T is the sum of all peak areas eluting within 30 min.

SUCROSE MONOESTERS OF LAURIC, PALMITIC OR STEARIC ACID
(TENTATIVE)

New tentative specifications prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010). A group ADI of 0 - 30 mg/kg bw for this substance together with sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II was established at the 73rd JECFA (2010).

Information is required on a test method to distinguish from sucrose esters of fatty acids.

Note: The tentative specifications will be withdrawn unless the requested information is received before the end of 2011.

DEFINITION

The product consists of sucrose monoesters of individual fatty acids, namely lauric, palmitic or stearic acid. They are manufactured by a transesterification reaction of sucrose and vinyl esters of lauric, palmitic or stearic acids in dimethyl sulfoxide. Impurities are removed by a series of evaporation and solvent extraction steps. Only the following solvents may be used for the production: dimethyl sulfoxide and isobutanol.

Assay

Total content of sucrose esters: not less than 85%
Content of monoesters: not less than 90% of total sucrose esters

DESCRIPTION

White to off white powder

FUNCTIONAL USES

Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4)

Soluble in water and in ethanol

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 waterbath 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.

PURITY

Sulfated ash (Vol.4)

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

Acid value (Vol.4)	Not more than 6
Free sucrose	Not more than 5% See description under TESTS
Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Isobutanol	Not more than 10 mg/kg See description under TESTS
Vinyl laurate, vinyl palmitate and vinyl stearate	Not more than 10 mg/kg See description under TESTS
Acetaldehyde	Not more than 1 mg/kg See description under TESTS
Lead (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

Free sucrose

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

Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 280 °
- column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
- 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:

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

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.
Carrier gas: Nitrogen
Flow rate: 30 ml/min
Detector: Flame photometric detector (using 394 nm sulfur filter)
Temperatures
- injection port: 210°
- column: 160°
The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3.4 min.
Note: Before using the column, 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 the column conditioning.

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 (C_{DMSO}) from:

$$C_{DMSO} \text{ (mg/kg)} = C \times 25 / W$$

where
C is dimethyl sulfoxide concentration determined (μg/ml); and
W is weight of sample (g).

Isobutanol,

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 of isobutanol by weighing accurately 0.2 g of isobutanolinto 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 1 g of the sample are equal to 20, 10 and 5 mg/kg of isobutanol, 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
Temperatures
- injection port: 110°
- column: 40°
- detector: 110°
Head space sampler:
- sample heat insulating temperature: 80°
- sample heat insulating period: 40 min
- syringe temperature: 85°
- sample gas injection: 1.0 ml

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

C_i (mg/kg)= w_i / W

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

Vinyl laurate, vinyl palmitate and vinyl stearate

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

Standard solutions
Prepare a stock solution separately containing 100.0 μg/ml of vinyl laurate, vinyl palmitate or vinyl stearate in acetonitrile. Prepare a range of mixed standard solutions containing 0.5, 1, 2 and 5 μg/ml of vinyl laurate, vinyl palmitate and vinyl stearate in acetonitrile.

Procedure
Weigh accurately 0.5 g of the sample into a 5-ml volumetric flask. Dilute to volume with methanol and mix using Vortex until the sample dissolves. Inject 1 μl of the sample solutions into the chromatograph.

Chromatography conditions
Column: Nitroterephthalic acid modified polyethylene glycol (DB-FFAP or equivalent) (30 m x 0.32 mm i.d. with 0.5 μm film)
Carrier gas: Nitrogen
Pressure: 7.18 psi
Split ratio: 10:1
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 230 °
- column: Hold for 4 min at 100°, then 100-230° at 45°/min, hold for 10 min at 230°
- detector: 250 °
The retention times of vinyl laurate, vinyl palmitate and vinyl stearate measured under the above conditions are approx. 9.1, 12.0 and 14.4 min, respectively.

Calculation
Calculate the content of vinyl laurate, vinyl palmitate and vinyl stearate from:

Content of vinyl laurate, vinyl palmitate and vinyl stearate (mg/kg) = C x 5 / W

where
C is concentration of vinyl laurate, vinyl palmitate and vinyl stearate determined (μg/ml); and
W is weight of sample (g).

Acetaldehyde

Principle
The volatile acetaldehyde is converted with an acidic solution of 2, 4- dinitrophenylhydrazine (DNPH) to a more stable compound, acetaldehyde-2, 4-dinitrophenylhydrazone (ADNPH) that absorbs

in the UV region. ADNPH is determined by HPLC under the following conditions.

Standard solutions
Prepare ADNPH stock solution of 40 μg/ml from ADNPH standard (Sigma) with acetonitrile. Prepare a range of solutions containing 0, 0.05, 0.1, 0.2 and 0.5 μg/ml of ADNPH by dilutions of the stock solution with acetonitrile.

Chromatography conditions
Column: NUCLEOSIL 100-5 C18 (250 mm x 4.6 mm i.d., 5 μm) or equivalent
Mobile phase: Methanol - 1.0mM LiCl solution (80:20)
Flow rate: 1.0 ml/min
Detector: UV 360 nm
Column temperatures: 40°
The retention time of ADNPH measured under the above conditions is approx. 20 min.

Procedure
Accurately weigh 0.5 g of the sample into a 5-ml volumetric flask. Add 1.5mL of methanol to dissolve the sample, add 0.5ml of DNPH reagent and make to volume with acetonitrile. Stir the mixture with a magnetic stirrer for 10min. Centrifuge and collect the liquid layer. Filter through a 0.45 μm membrane filter. Inject 20 μl of the sample solution into HPLC.

Calculation
Calculate the content of acetaldehyde from:

$$\text{Content of acetaldehyde (mg/kg)} = C \times 5 / W$$

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

METHOD OF ASSAY

Determine by HPLC using 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 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:
- Column: 40°
- Detector: 40°

Record the chromatogram for about 50 min.

Calculate the percentage of total sucrose esters in the sample from:

$$\% \text{ sucrose esters} = 100 (A_m+A_d) / T$$

Calculate the percentage of monoesters in total sucrose esters from:

$$\% \text{ monoesters} = 100 A_m / (A_m+A_d)$$

where
A_m is the peak area of the monoesters eluting at about 39.0-40.0 min;
A_d is the peak area of the diesters eluting at about 37.0-38.2 min; and
T is the sum of all peak areas eluting within 50 min.

TITANIUM DIOXIDE

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). An ADI "not limited" was established at the 13th JECFA (1969).

SYNONYMS Titania; CI Pigment white 6; CI (1975) No. 77891; INS No. 171

DEFINITION

Titanium dioxide is produced by either the sulfate or the chloride process. Processing conditions determine the form (anatase or rutile structure) of the final product.

In the sulfate process, sulfuric acid is used to digest ilmenite ($FeTiO_3$) or ilmenite and titanium slag. After a series of purification steps, the isolated titanium dioxide is finally washed with water, calcined, and micronized.

In the chloride process, chlorine gas is reacted with a titanium-containing mineral under reducing conditions to form anhydrous titanium tetrachloride, which is subsequently purified and converted to titanium dioxide either by direct thermal oxidation or by reaction with steam in the vapour phase. Alternatively, concentrated hydrochloric acid can be reacted with the titanium-containing mineral to form a solution of titanium tetrachloride, which is then further purified and converted to titanium dioxide by hydrolysis. The titanium dioxide is filtered, washed, and calcined.

Commercial titanium dioxide may be coated with small amounts of alumina and/or silica to improve the technological properties of the product.

C.A.S. number 13463-67-7

Chemical formula TiO_2

Formula weight 79.88

Assay Not less than 99.0% on the dried basis (on an aluminium oxide and silicon dioxide-free basis)

DESCRIPTION White to slightly coloured powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, hydrochloric acid, dilute sulfuric acid, and organic solvents. Dissolves slowly in hydrofluoric acid and hot concentrated sulfuric acid.

Colour reaction	Add 5 ml sulfuric acid to 0.5 g of the sample, heat gently until fumes of sulfuric acid appear, then cool. Cautiously dilute to about 100 ml with water and filter. To 5 ml of this clear filtrate, add a few drops of hydrogen peroxide; an orange-red colour appears immediately.
PURITY	
Loss on drying (Vol. 4)	Not more than 0.5% (105°, 3 h)
Loss on ignition (Vol. 4)	Not more than 1.0% (800°) on the dried basis
Aluminium oxide and/or silicon dioxide	Not more than 2%, either singly or combined See descriptions under TESTS
Acid-soluble substances	Not more than 0.5%; Not more than 1.5% for products containing alumina or silica. Suspend 5 g of the sample in 100 ml 0.5 N hydrochloric acid and place on a steam bath for 30 min with occasional stirring. Filter through a Gooch crucible fitted with a glass fibre filter paper. Wash with three 10-ml portions of 0.5 N hydrochloric acid, evaporate the combined filtrate and washings to dryness, and ignite at a dull red heat to constant weight.
Water-soluble matter (Vol. 4)	Not more than 0.5% Proceed as directed under acid-soluble substances (above), using water in place of 0.5 N hydrochloric acid.
Impurities soluble in 0.5 N hydrochloric acid	
Antimony	Not more than 2 mg/kg See description under TESTS
Arsenic	Not more than 1 mg/kg See description under TESTS
Cadmium	Not more than 1 mg/kg See description under TESTS
Lead	Not more than 10 mg/kg See description under TESTS
Mercury (Vol. 4)	Not more than 1 mg/kg Determine using the cold vapour atomic absorption technique. Select a sample size appropriate to the specified level

TESTS

PURITY TESTS

Impurities soluble in 0.5 N hydrochloric acid	
Antimony, arsenic, cadmium and lead (Vol.4)	Transfer 10.0 g of sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat to boiling on a hot plate. Boil gently for 15 min, pour the slurry into a 100- to 150-ml centrifuge bottle, and centrifuge for 10 to 15 min, or until

undissolved material settles. Decant the supernatant through Whatman No. 4 filter paper, or equivalent, collecting the filtrate in a 100-ml volumetric flask and retaining as much as possible of the undissolved material in the centrifuge bottle. Add 10 ml of hot water to the original beaker, washing off the watch glass with the water, and pour the contents into the centrifuge bottle. Form a slurry, using a glass stirring rod, and centrifuge. Decant through the same filter paper, and collect the washings in the volumetric flask containing the initial extract. Repeat the entire washing process two more times. Finally, wash the filter paper with 10 to 15 ml of hot water. Cool the contents of the flask to room temperature, dilute to volume with water, and mix.

Determine antimony, cadmium, and lead using an AAS/ICP-AES technique appropriate to the specified level. Determine arsenic using atomic absorption hydride technique.

Aluminium oxide

Reagents and sample solutions

Ammonium acetate buffer solution
In a 1000-ml volumetric flask, dissolve 77 g of ammonium acetate in about 500 ml of water, add 10 ml of glacial acetic acid and dilute to volume with water.

Diammonium hydrogen phosphate solution
In a 1000-ml volumetric flask, dissolve 150 g of diammonium hydrogen phosphate in about 700 ml of water, adjust pH to 5.5 using a 1 in 2 solution of hydrochloric acid, then dilute to volume with water.

Zinc Sulfate solution (0.01 N)
Dissolve 2.9 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in sufficient water and make up to 1000 ml in a volumetric flask. Standardize the solution as follows: Dissolve 500 mg of high-purity (99.9%) aluminium wire, accurately weighed, in 20 ml of concentrated hydrochloric acid, heating gently to effect solution, then transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix. Transfer a 10 ml aliquot of this solution into a 500 ml Erlenmeyer flask containing 90 ml of water and 3 ml of concentrated hydrochloric acid, add 1 drop of methyl orange TS and 25 ml of 0.02 M disodium ethylenediaminetetraacetate (EDTA) Add, dropwise, ammonia solution (1 in 5) until the colour is just completely changed from red to orange-yellow. Then, add 10 ml of ammonium acetate buffer solution and 10 ml of diammonium hydrogen phosphate solution. Boil the solution for 5 min, cool it quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix.

Using zinc sulfate solution as titrant, titrate the solution to the first yellow-brown or pink end-point colour that persists for 5-10 sec. (NOTE: This titration should be performed quickly near the end-point by adding rapidly 0.2 ml increments of the titrant until the first colour change occurs; although the colour will fade in 5-10 sec, it is the true end-point. Failure to observe the first colour change will result in an incorrect titration. The fading end-point does not occur

at the second end-point)

Add 2 g of sodium fluoride, boil the mixture for 2-5 min, and cool in a stream of running water. Titrate this solution, using the zinc sulfate solution as titrant, to the same fugitive yellow-brown or pink end-point as described above.

Calculate mass (mg) of Al_2O_3 per ml of zinc sulfate solution (T) from the formula

$$T = 18.896\ W/V$$

where

W is the mass (g) of aluminium wire;
V is the ml of the zinc sulfate solution consumed in the second titration;
18.896 = (R × 1000 mg/g × 10 ml/2)/1000 ml; and
R is the ratio of the formula weight of aluminium oxide to that of elemental aluminium.

Sample Solution A

Accurately weigh 1.0 g of the sample and transfer to a 250-ml high-silica glass Erlenmeyer flask. Add 10 g of sodium bisulfate ($NaHSO_4 \cdot H_2O$). (*Note*: Do not use more sodium bisulfate than specified, as an excess concentration of salt will interfere with the EDTA titration later on in the procedure.) Begin heating the flask at low heat on a hot plate, and then gradually raise the temperature until full heat is reached. (*Caution:* perform this procedure in a well ventilated area) When spattering has stopped and light fumes of SO_3 appear, heat in the full flame of a Meeker burner, with the flask tilted so that the fusion of the sample and sodium bisulfate is concentrated at one end of the flask. Swirl constantly until the melt is clear (except for silica content), but guard against prolonged heating to avoid precipitation of titanium dioxide. Cool, add 25 ml sulfuric acid solution (1 in 2), and heat until the mass has dissolved and a clear solution results. Cool, and dilute to 120 ml with water. Introduce a magnetic stir bar into the flask.

Sample Solution B

Prepare 200 ml of an approximately 6.25 M solution of sodium hydroxide. Add 65 ml of this solution to Sample Solution A, while stirring with the magnetic stirrer; pour the remaining 135 ml of the alkali solution into a 500-ml volumetric flask.

Slowly, with constant stirring, add the sample mixture to the alkali solution in the 500-ml volumetric flask; dilute to volume with water, and mix. (*Note*: If the procedure is delayed at this point for more than 2 hours, store the contents of the volumetric flask in a polyethylene bottle.) Allow most of the precipitate to settle (or centrifuge for 5 min), then filter the supernatant liquid through a very fine filter paper. Label the filtrate Sample Solution B.

Sample Solution C

Transfer 100 ml of the Sample Solution B into a 500-ml Erlenmeyer flask, add 1 drop of methyl orange TS, acidify with hydrochloric acid solution (1 in 2), and then add about 3 ml in

excess. Add 25 ml of 0.02 M disodium EDTA, and mix. [*Note*: If the approximate Al_2O_3 content is known, calculate the optimum volume of EDTA solution to be added by the formula: (4 x % Al_2O_3) + 5 ml]

Add, dropwise, ammonia solution (1 in 5) until the colour is just completely changed from red to orange-yellow. Then add10 ml each of ammonium acetate and diammonium hydrogen phosphate solution and boil for 5 min. Cool quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix. If the solution is purple, yellow-brown, or pink, bring the pH to 5.3 - 5.7 by the addition of acetic acid. At the desired pH, a pink colour indicates that not enough of the EDTA solution has been added, in which case, discard the solution and repeat this procedure with another 100 ml of Sample Solution B, using 50 ml, rather than 25 ml, of 0.02 M disodium EDTA.

Procedure

Using the standardized zinc sulfate solution as titrant, titrate Sample Solution C to the first yellow-brown or pink end-point that persists for 5-10 sec. (*Important:* See Note under "*0.01 Zinc sulfate*"). This first titration should require more than 8 ml of titrant, but for more accurate work a titration of 10-15 ml is desirable.

Add 2 g of sodium fluoride to the titration flask, boil the mixture for 2-5 min, and cool in a stream of running water. Titrate this solution, using the standardized zinc sulfate solution as titrant, to the same fugitive yellow-brown or pink end-point as described above.

Calculation

Calculate the percentage of aluminium oxide (Al_2O_3) in the sample taken by the formula:

$$\% Al_2O_3 = 100 \times (0.005VT)/S$$

where

V is the number of ml of 0.01 N zinc sulfate consumed in the second titration;
T is the mass of Al_2O_3 per ml of zinc sulfate solution;
S is the mass (g) of the sample taken; and
0.005 = 500 ml / (1000mg/g × 100 ml).

Silicon dioxide

Accurately weigh 1 g of the sample and transfer to a 250-ml high-silica glass Erlenmeyer flask. Add 10 g of sodium bisulfate ($NaHSO_4 \cdot H_2O$). Heat gently over a Meeker burner, while swirling the flask, until decomposition and fusion are complete and the melt is clear, except for the silica content, and then cool. (*Caution:* Do not overheat the contents of the flask at the beginning, and heat cautiously during fusion to avoid spattering.)

To the cooled melt add 25 ml of sulfuric acid solution (1 in 2) and heat carefully and slowly until the melt is dissolved. Cool, and carefully add 150 ml of water by pouring very small portions down the sides of the flask, with frequent swirling to avoid over-heating and spattering. Allow the contents of the flask to cool, and filter through fine ashless filter paper, using a 60 degree gravity funnel.

Rinse out all the silica from the flask onto the filter paper with sulfuric acid solution (1 in 10). Transfer the filter paper and its contents into a platinum crucible, dry in an oven at 120°, and heat the partly covered crucible over a Bunsen burner. To prevent flaming of the filter paper, first heat the cover from above, and then the crucible from below.

When the filter paper is consumed, transfer the crucible to a muffle furnace and ignite at 1000° for 30 min. Cool in a desiccator, and weigh. Add 2 drops of sulfuric acid (1 in 2) and 5 ml of concentrated hydrofluoric acid (sp.gr. 1.15), and carefully evaporate to dryness, first on a low-heat hot plate (to remove the HF) and then over a Bunsen burner (to remove the H_2SO_4). Take precautions to avoid spattering, especially after removal of the HF. Ignite at 1000^o for 10 min, cool in a desiccator, and weigh again. Record the difference between the two weights as the content of SiO_2 in the sample.

METHOD OF ASSAY

Accurately weigh about 150 mg of the sample, previously dried at 105° for 3 hours, and transfer into a 500-ml conical flask. Add 5 ml of water and shake until a homogeneous, milky suspension is obtained. Add 30 ml of sulfuric acid and 12 g of ammonium sulfate, and mix. Initially heat gently, then heat strongly until a clear solution is obtained. Cool, then cautiously dilute with 120 ml of water and 40 ml of hydrochloric acid, and stir. Add 3 g of aluminium metal, and immediately insert a rubber stopper fitted with a U-shaped glass tube while immersing the other end of the U-tube into a saturated solution of sodium bicarbonate contained in a 500-ml wide-mouth bottle, and generate hydrogen. Allow to stand for a few minutes after the aluminium metal has dissolved completely to produce a transparent purple solution. Cool to below 50° in running water, and remove the rubber stopper carrying the U-tube. Add 3 ml of a saturated potassium thiocyanate solution as an indicator, and immediately titrate with 0.1 N ferric ammonium sulfate until a faint brown colour that persists for 30 seconds is obtained. Perform a blank determination and make any necessary correction. Each ml of 0.1 N ferric ammonium sulfate is equivalent to 7.990 mg of TiO_2.

WITHDRAWAL OF SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

Annatto extract (oil-processed bixin)

In the call for data for the 73rd meeting information was requested to revise the existing tentative specifications, stating that the specifications would be withdrawn if no information was forthcoming.

As no supporting information was received, the tentative specifications were withdrawn by the Committee.