SUCROSE MONOESTERS OF LAURIC, PALMITIC OR STEARIC ACID

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding tentative specifications prepared at the 73rd JECFA (2010), 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).

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.

Composition of fatty acids

The chromatogram obtained by following the procedure in TESTS represents mainly one peak, which corresponds to methyl laurate, methyl palmitate or methyl stearate.

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

IDENTIFICATION TESTS

Composition of fatty acids

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

Standard solutions

Dissolve 0.1 g each of methyl laurate, methyl palmitate and methyl stearate in heptane and dilute to 50.0 ml with heptane.

Chromatography conditions

Column: DB-WAX (10-30 m x 0.2-0.8 mm i.d. with 0.1-1.0 µm film) or equivalent
Carrier gas: Helium or hydrogen
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 250°
- column: 200°
- detector: 250°

The peaks of methyl laurate, methyl palmitate and methyl stearate are separate under the conditions.

Procedure

Weigh 0.15 g of the sample and dissolve in 2 ml of a 20 g/l sodium hydroxide-methanol solution in a 25 ml pear-shape flask. Boil under a reflux condenser for 30 min. Add 2 ml of 14% boron trifluoride-methanol solution through the condenser and boil for 30 min. Add 4 ml heptane through the condenser and boil for 5 min. Cool and add 10 ml saturated sodium chloride solution and shake for 15 s. Add saturated sodium chloride solution to bring the upper phase into the neck of the flask. Collect 2 ml of the upper phase, wash 3 times with each 2 ml of water and dry over anhydrous sodium sulphate. Inject 1 µl into the chromatograph.

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 diluting 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 volume.

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 of each standard solution in place of the sample and *N,N*-dimethylformamide. Draw a standard curve by plotting the concentration of sucrose in 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 using the following formula:

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

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 by injecting 3 μl of each standard solution into the chromatograph.

Calculate the concentration of dimethyl sulfoxide in mg/kg (C_{DMSO}) using the following formula:

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

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 isobutanol 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 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) using the following formula:

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

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 separate stock solutions each 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 using the following formula:

$$\text{Content of vinyl laurate, vinyl palmitate and vinyl stearate (mg/kg)} = C \times 5 / W$$

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 using the following formula:

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

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 using the following formula:

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

Calculate the percentage of monoesters in total sucrose esters using the following formula:

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

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 43 min.