SUCROSE ESTERS OF FATTY ACIDS

(TENTATIVE)

Information required on
- *method of analysis for the determination of free sucrose using capillary GC or HPLC*
- *an alternative and less toxic solvent than pyridine for preparing the standard and sample solutions for the determinations of free sucrose and propylene glycol*
- *method of analysis for the determination of dimethyl sulfoxide that does not require a packed column*

Note: The tentative specifications will be withdrawn unless the requested information is received before the end of the year 2006.

Tentative specifications prepared at the 65th JECFA (2005) and published in FNP 52 Add 13 (2005), superseding the specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). An ADI of 0-30 mg/kg bw for this substance together with sucroglycerides was established at the 49th JECFA (1997).

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 Stiff gels, soft solids or white to slightly greyish white powders

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 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.

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 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 methods described in Volume 4, "Instrumental methods".

TESTS

PURITY TESTS

Free sucrose

Determine by gas liquid chromatography (Volume 4).

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 pyridine.

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 pyridine to the mark.

Chromatography conditions

Column: 2% Dexsil 300GC on Uniport HP 80/100 mesh (slightly polar, 2.1 m x 3.2 mm i.d.) or equivalent
Carrier gas: Nitrogen
Flow rate: 40 ml/min
Detector: FID
Temperatures:
- injection: 280 °

- column: Hold for 1 min at 160°, then 160-300° at 15°/min, hold for 60 min at 300 °
- detector: 320 °
The retention times of free sucrose and octacosane measured under the above conditions are approx. 8.2 and 9.8 min, respectively.

Procedure
Weigh accurately 20-50 mg of the sample into a centrifugation tube, add 1 ml internal standard solution, 1 ml pyridine, 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 gas liquid chromatograph.

Standard curve
Prepare silylate standard solutions following the same procedure using 1 ml each of the standard solutions in place of the sample and pyridine. 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 in sample from the standard curve.

Calculate the percentage of free sucrose from:

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

Dimethylformamide

Determine by gas liquid chromatography (Volume 4).

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 (NPD) (synonym: Flame thermoionic detector (FTD))
Temperatures:
- injection: 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 C_{DFA} of dimethylformamide from:

C_{DFA} (mg/kg) = [C (μg/ml) x 20 (ml)] / W (g)

where
C = dimethylformamide concentration detected (μg/ml)
W = weight of sample (g)

Note: The nitrogen phosphorus detector is insensitive to components that do not contain nitrogen or phosphorus. As a consequence, the capillary column can become obstructed with compounds of low volatility, although the baseline of the chromatogram is stable. Accordingly, the column must be reconditioned frequently. Overnight reconditioning (flow carrier gas in the reverse direction at 180°) is required after about every 15 samples.

Dimethyl sulfoxide

Determine by gas liquid chromatography (Vol. 4).

Standard solutions
Prepare a 0.25 mg/ml stock solution of dimethyl sulfoxide in tetrahydrofuran. Prepare a range of solutions containing 0.5, 1 and 5 μg/ml of dimethyl sulfoxide by dilutions of the stock solution with tetrahydrofuran.

Chromatography conditions
Column: 10% PEG 20M and 3% KOH on Gas Chrom Z (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: 50 ml/min
Detector: Flame photometric detector (using 394 nm sulfur filter)
Temperatures
- injection: 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 C_{DMSO} of dimethyl sulfoxide in mg/kg from:

C_{DMSO} (mg/kg) = [C (μg/ml) x 25 (ml)] / W (g)

where
C = dimethyl sulfoxide concentration determined (μg/ml)
W = weight of sample (g)

Propylene glycol

Determine by gas liquid chromatography (Vol. 4).

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

Standard solution
Prepare a 50 μg/ml solution of propylene glycol in pyridine.

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: 230°
- column: Hold for 5 min at 60°, then 60-250° at 20°/min, hold for 5 min at 250°
- detector: 250°
The retention times of ethylene glycol and propylene glycol derivatives are approx. 7.7 min and 7.9 min, respectively.

Procedure
Weigh accurately 1 g of the sample in a 10-ml volumetric flask, and add 100 μl of the internal standard solution. Dissolve and make to volume with pyridine. Take 0.5 ml of 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 0.05, 0.2, 0.5 and 1 ml of the standard solution in place of the sample.

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

C_{PG} (mg/kg) = [C (μg/ml) x 10 (ml)] / W (g)

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

Note: It will be necessary to clean the injection port and to recondition column at 300° after about every 20 samples, because of contamination of the column.

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

Determined by gas chromatography with a head space sampler.

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 in 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, for example DB-1 manufactured by J&W Co. Ltd.)
Carrier gas: Nitrogen
Flow rate: 3.5 ml/min
Detector: FID
Temperatures
- injection: 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 each solvent using the analytical results. The relationship should be linear ($R^2 > 0.99$). Extrapolate and determine the x-intercept, w_i, and calculate the solvent concentrations C_i in mg/kg in the sample from:

$$C_i = w_i / W$$

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

METHOD OF ASSAY Determine by HPLC using the following conditions:

Procedure

Accurately weigh 250 mg of the sample and transfer to a 50-ml volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.5 μm membrane filter. Inject 100 μl of the sample into the pre-stabilized chromatograph.

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G2000 (Tosoh) or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 0.7 ml/min
Detector: RI
Temperatures:
- Column: 38°
- Detector: 38°
Record the chromatogram for about 90 min.

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

% sucrose ester = 100 A/T

where
A = the sum of peak areas for the three main components, the mono-, di- and tri-esters, eluting at about 65, 68 and 73 min, respectively
T = the sum of all peak areas eluting within 90 min