

## SUCROSE OLIGOESTERS TYPE I

*New specifications prepared at the 71<sup>st</sup> JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI of 0 - 30 mg/kg bw for sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II was established at the 71<sup>st</sup> JECFA (2009).*

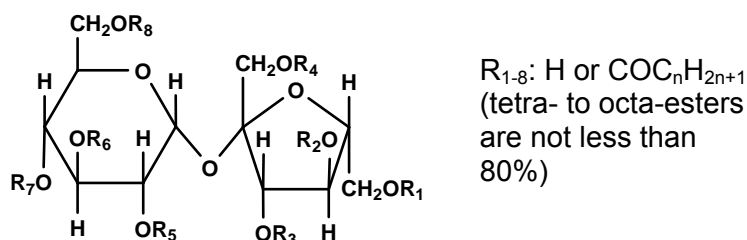
### SYNONYMS

Sucrose fatty acid esters (high-esterified); Sucrose oligoesters (high-esterified); INS No. 473a

### DEFINITION

Sucrose oligoesters type I contains mainly tetra- to octa-fatty acid esters of sucrose, though the content of hepta- and octa-esters is not more than 50%. They are prepared from sucrose and methyl esters of food fatty acids such as stearic acid, palmitic acid, oleic acid, lauric acid and erucic acid by interesterification in the presence of an alkaline catalyst. Only the following solvents may be used for the production: dimethyl sulfoxide, isobutanol and methyl ethyl ketone.

Structural formula



Assay

Total content of tetra- to octa-esters: not less than 80%  
Content of hepta- and octa-esters: not more than 50%  
Content of octa-esters: not more than 20%

### DESCRIPTION

White to red-brown powders, soft solid, stiff gels or colourless to red-brown viscous liquid

### FUNCTIONAL USES

Emulsifier, stabilizer, tableting aid

### CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4)

Insoluble in water

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 is 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

<u>Sulfated ash</u> (Vol.4)	Not more than 2% Test 1 g of the sample (Method I)
<u>Acid value</u> (Vol.4)	Not more than 6
<u>Free sucrose</u>	Not more than 1 % See description under TESTS
<u>Dimethyl sulfoxide</u>	Not more than 2 mg/kg See description under TESTS
<u>Isobutanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methyl ethyl ketone</u>	Not more than 10 mg/kg See description under TESTS
<u>Lead</u> (Vol.4)	Not more than 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 Volume 4 (under "General Methods, Metallic Impurities").

## TESTS

### PURITY TESTS

Free sucrose Determine by gas liquid chromatography described in Volume 4 using 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: 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 gas liquid 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:

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

#### Dimethyl sulfoxide

Determine by gas liquid chromatography described in Volume 4 using 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: 210°

- column: 160°

The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3 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 ( $C_{\text{DMSO}}$ ) from:

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

where

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

### Methanol, isobutanol, and methyl ethyl ketone

Determined by gas chromatography with a head space sampler using the following methods.

### Standard solutions

Prepare standard solution A containing 4000 mg/l each of methanol, isobutanol, 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: 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. Extrapolate and determine the x-intercept ( $w_i$ ), and calculate the solvent concentrations ( $C_i$ ) in the sample from:

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

where

$w_i$  is the x-intercept of relationship line using the standard addition

method ( $\mu\text{g}$ ); and  
W is the weight of sample (g).

## METHOD OF ASSAY 1. Tetra- to octa-esters

Determine by HPLC using the following conditions:

### Procedure

Accurately weigh 250 mg of the sample into a 100-ml volumetric flask. Dissolve and dilute to volume with tetrahydrofuran and mix. Filter through a 0.5  $\mu\text{m}$  membrane filter. Inject 80  $\mu\text{l}$  of the sample into the 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  $\mu\text{m}$  particles for solvent manufactured by Tosoh in series or equivalent)

Mobile phase: HPLC-grade tetrahydrofuran

Flow rate: 0.8 ml/min

Detector: RI

Temperatures:

- Column: 40°

- Detector: 40°

Record the chromatogram for about 50 min

### Identification of the peaks

More highly esterified components elute earlier and tetra- to octa-esters elute as one peak. Their retention times are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these 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. The retention time (min) of mono-, di-, tri- and tetra- to octa-esters esterified with main fatty acids

Esterified fatty acid	Mono-esters	Di-esters	Tri-esters	Tetra- to octa-esters
Lauric acid	40.0	38.2	37.0	36.2
Palmitic acid	39.3	37.2	36.0	35.1
Stearic acid	39.0	37.0	35.7	34.9
Oleic acid	39.1	37.1	35.9	35.0
Erucic acid	38.5	36.3	35.1	34.3

Calculate the percentage of tetra- to octa-esters ( $E_{\text{tet-oct}}$ ) from:

$$E_{\text{tet-oct}}(\%) = 100 A_{\text{tet-oct}}/T$$

where

$A_{\text{tet-oct}}$  is the sum of peak areas for tetra- to octa-esters; and

T is the sum of all peak areas eluting within 43 min.

## 2. Hepta- and octa-esters

The percentage of the sum of hepta- and octa-esters ( $E_{\text{hep+oct}}$ ), and the percentage of octa-esters ( $E_{\text{oct}}$ ) is calculated by two steps. The ratio of hepta- and octa-esters, and the ratio of octa-esters in sum of

tetra- to octa-esters are determined by HPLC. Then  $E_{\text{hep+oct}}$  and  $E_{\text{oct}}$  are calculated using  $E_{\text{tet-oct}}$  obtained above in Method 1.

### Procedure

Accurately weigh 1g of the sample into a 50-ml volumetric flask and add a solution for the mobile phase (tetrahydrofuran/methanol=50/50 (vol/vol)) to the mark. Filter through a 0.5  $\mu\text{m}$  membrane filter. Inject 20  $\mu\text{l}$  of the sample into the chromatograph.

### Chromatography conditions

Column: reversed phase C18 columns (150mm x 4.6 mm i.d.; ODS-2 manufactured by GL Science or equivalent)

Mobile phase: tetrahydrofuran/methanol=50/50 (vol/vol)

Flow rate: 0.8 ml/min

Detector: RI

Temperatures:

- Column: 40°

- Detector: 40°

Record the chromatogram for about 16 min

### Identification of the peaks

The retention times of tetra-, penta-, hexa-, hepta- and octa-esters are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 2. 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 2. The retention time (min) of tetra- to octa-esters esterified with main fatty acids

Esterified fatty acid	Tetra-esters	Penta-esters	Hexa-esters	Hepta-esters	Octa-esters
Lauric acid	2.5	2.7	3.0	3.4	4.0
Palmitic acid	2.9	3.5	4.3	5.5-5.9*	7.5-9.3*
Stearic acid	3.1	3.7	4.8	6.1-7.0*	7.9-10.7*
Oleic acid	2.8	3.2	3.8	4.7	5.4-6.7*
Erucic acid	3.3	4.1	5.4	7.5	11.0

\*: the retention time range, because highly esterified components have been shown as several unresolved peaks

Calculate the percentage of the sum of hepta- and octa-esters ( $E_{\text{hep+oc}}$ ) and the percentage of octa-esters ( $E_{\text{oct}}$ ) as follows:

$$E_{\text{hep+oc}}(\%) = (B_{\text{hep+oc}}/T_{\text{tet-oct}}) \times E_{\text{tet-oct}}$$

$$E_{\text{oct}}(\%) = (B_{\text{oct}}/T_{\text{tet-oct}}) \times E_{\text{tet-oct}}$$

where

$B_{\text{hep+oc}}$  is the sum of peak areas of hepta-esters and octa-esters;

$B_{\text{oct}}$  is the peak areas for octa-esters;

$T_{\text{tet-oct}}$  is the sum of peak areas from tetra- to octa-esters; and

$E_{\text{tet-oct}}$  (%) is the percentage of tetra- to octa-esters measured by Method 1 described above.