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CITRIC and FATTY ACID ESTERS of GLYCEROL

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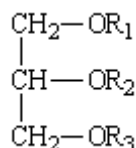
CITRIC and FATTY ACID ESTERS of GLYCEROL

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SYNONYMS Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

DEFINITION Citric and fatty acid esters of glycerol (CITREM) consists of mixed esters of citric acid and edible fatty acids with glycerol. CITREM is obtained by esterification of glycerol with citric acid and edible fatty acids, or by reaction of a mixture of mono- and diglycerides of edible fatty acids with citric acid. It may contain minor amounts of free fatty acids, free glycerol, free citric acid and mono- and diglycerides. The mono- and diglycerides may include either one or two edible fatty acids from C12:0 to C18:0, mainly palmitic (C16:0) and stearic (C18:0) acids. It may also contain minor amounts of other fatty acids such as myristic (C14:0), oleic (C18:1), linoleic (C18:2) and arachidic acid (C20:4). CITREM may be partially or wholly neutralized with sodium hydroxide or potassium hydroxide or by using sodium, potassium or calcium salts of weak acids such as acetic, lactic, propionic or carbonic acids.

Structural formula



Where at least one of R₁, R₂ or R₃ represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION White to ivory coloured, oily to waxy material.

FUNCTIONAL USES Stabilizer, emulsifier, dough conditioner, antioxidant synergist.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in oils and fats; insoluble in ethanol.

Test for fatty acids (Vol. 4) Passes test

Test for citric acid (Vol. 4) Passes test

Test for glycerol Passes test

(Vol. 4)

PURITY

Sulfated ash (Vol. 4) Non-neutralized products: not more than 0.5%
Partially neutralized products: not more than 3%
Wholly neutralized products: not more than 10%
Test 2 g of the sample (Method I).

Free glycerol (Vol. 4) Not more than 4%

Total glycerol 8-33%
See description under TESTS

Total citric acid 13-50%
See description under TESTS

Total fatty acid 37-81%
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg.
(Not more than 0.5 mg/kg for use in infant formula and formula for special medical purposes intended for infants).

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Total glycerol Total glycerol, after hydrolysis with KOH, is determined by HPLC (Vol. 4)

Equipment and Reagents:

HPLC consisting of a Refractive Index (RI) detector, autosampler and column thermostat.

Column: Aquasil C18 (250 mm x 4.6 mm x 5 µm) or equivalent

Glycerol standard: > 99.5%

Phosphoric acid: 85%

Potassium hydroxide: reagent grade

Potassium dihydrogen phosphate: reagent grade

Methanol, acetonitrile and water: HPLC grade

Preparation of standard solutions:

Prepare five solutions of glycerol by weighing 20 – 100 mg, to the nearest 0.01 mg, dissolving in water and making up to 100 ml in separate volumetric flasks.

Sample preparation:

Weigh, to the nearest 0.01 mg, about 50 mg of sample into a 25 ml screw cap vial. Add 2 ml of methanolic KOH (0.5 mol/l, prepared by weighing 33 g KOH into a 1-liter volumetric flask and diluting to volume with methanol). Hydrolyze for 2 hours in a heating block at 110°. Allow to cool, add 20 ml of dilute phosphoric acid (prepared by dissolving 5 ml phosphoric acid in 1 l of water) and shake well. Remove 500 µl of the aqueous phase and filter through 0.45 µm membrane filter for HPLC analysis.

Procedure:

Mobile phase: Dissolve 6.8 g KH₂O₄ in a 1 l volumetric flask with water, add 5 ml phosphoric acid (85%) and make up to volume with water.

Column temperature: 35°

RI detector temperature: 35°

Flow rate: 1.0 ml/min

Injection volume: 10 µl

Inject separately 10 µl of each standard solution and construct the standard curve. The retention time for glycerol is about 2.6 min. Inject 10 µl sample solution and calculate the percentage of glycerol in the sample as below:

Calculation

Determine the content of glycerol using the standard curve. Calculate the total glycerol by the following equation.

$$\text{Total glycerol, \%w/w} = (\text{CU (mg/ml)} * 22 \text{ (ml)} * 100) / W$$

W = weight of sample, mg

CU = concentration of glycerol determined from the standard curve (mg/ml).

Total citric acid

Total citric acid after hydrolysis is determined by HPLC.

Equipment and Reagents:

HPLC consisting of an Ultraviolet (UV) detector, autosampler and a column thermostat.

Column: Synergi 4 µm Hydro RP 80 A (4.6 mm x 250 mm) or equivalent.

Citric acid standard: > 99.5%

Phosphoric acid: 85%

Potassium hydroxide: reagent grade

Methanol, acetonitrile, KH₂PO₄ and water: HPLC grade

Preparation of standard solutions:

Prepare six solutions of citric acid by weighing 10-85 mg, to the nearest 0.01 mg, dissolving in neutralization solution (5 ml phosphoric acid, 85% in 1 l of HPLC water) and making up to 100 ml in separate volumetric flasks.

Sample preparation:

Weigh to the nearest 0.01 mg about 50 mg of sample into a 25 ml screw cap vial. Add 2 ml of methanolic KOH 0.5 mol/l (prepared by weighing 33 g KOH into a 1-liter volumetric flask and making to volume with methanol). Hydrolyze for 2 h in a heating block at 110°. Allow to cool, add 20 ml of dilute phosphoric acid (prepared by dissolving 5 ml phosphoric acid, in 1 l of water) and shake well. Filter 500 µl of the aqueous phase through a 0.45 µm membrane filter.

Procedure:

Mobile phase: Dissolve 6.8 g KH_2PO_4 in a 1 l volumetric flask with water, add 5 ml phosphoric acid (85%) and make up to volume with water.

Column temperature: 25°

UV detector at 205 nm

Flow rate: 1.0 ml/min

Injection volume: 20 µl

Inject separately 10 µl of each standard solution and construct the standard curve. The retention time of citric acid is about 6.9 min. Inject 10 µl of sample solution and calculate the percentage of citric acid in the sample as below:

Calculation

Determine the content of citric acid using the standard curve. Calculate the total citric acid by the following equation.

$$\text{Total Citric acid, \%w/w} = (\text{CU (mg/ml)} * 22 \text{ (ml)} * 100\%) / W$$

W = weight of the sample, mg

CU = concentration of the citric acid determined from the standard curve (mg/ml)

Total fatty acid

Principle: This method measures total fatty acids by extracting with diethyl ether.

Procedure

Weigh accurately 5.000 g of the sample into a 250-ml round-bottomed flask, add 50 ml of potassium hydroxide, ethanolic, TS, and reflux for 1 h on a water bath.

Quantitatively transfer the content of the saponification flask to a 1,000-ml separating funnel, using three 25-ml portions of water, and add 5 drops of methyl orange indicator solution.

Cautiously add 50% hydrochloric acid until the colour of solution changes to a red methyl orange end point. Add 1 ml of excess acid after the end point is reached. Shake well to mix the contents and separate the fatty acids.

Cool to room temperature and extract the separated fatty acids with three 100-ml portions of diethyl ether. Combine the extracts, and wash with 50-ml portions of 10% sodium chloride solution until the washed sodium chloride solution becomes neutral.

Dry the ether solution with anhydrous sodium sulfate. Then evaporate off ether on a steam bath, leave additional 10 min on the steam bath, and weigh the residue. This is the weight of the total fatty acids.

Calculation:

$$\text{Total Fatty acids, \%} = \frac{\text{mass of fatty acids, g} \times 100}{\text{mass of sample, g}}$$