

CYCLOTETRAGLUCOSE

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SYNONYMS

Cyclotetraose; Cyclic nigerosyl-(1→6)-nigerose; cycloalternan; cycloalternanotetraose; INS No. 1504(i)

DEFINITION

Cyclotetraglucose has been found to occur naturally in sake lees (i.e., the sediment that forms during sake production), in sake itself, and in the cells of *Saccharomyces cerevisiae*. It is a non-reducing cyclic tetrasaccharide consisting of four D-glucopyranosyl units linked by alternating $\alpha(1\rightarrow3)$ and $\alpha(1\rightarrow6)$ glycosidic bonds. It is produced from hydrolyzed food-grade starch by the actions of a mixture of 6- α -glucosyltransferase α -isomaltosyltransferase derived from *Sporosarcina globispora*, and cyclodextrin glucosyltransferase derived from *Bacillus stearothermophilus*. After purification the product contains 0 to 5 molecules of water of crystallization per molecule of cyclotetraglucose.

Chemical names

cyclo[$\rightarrow 6$]- α -D-glucopyranosyl-(1 \rightarrow 3)- α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-glucopyranosyl-(1 \rightarrow 3)- α -D-glucopyranosyl-(1 \rightarrow]

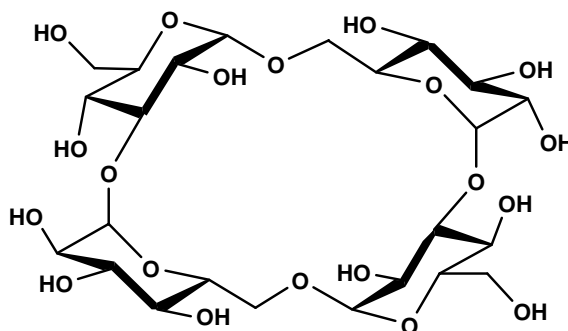
C.A.S. number

Cyclotetraglucose, anhydrous: 159640-28-5
Cyclotetraglucose, monohydrate: 532945-75-8
Cyclotetraglucose, pentahydrate: 532945-76-9

Chemical formula

C₂₄H₄₀O₂₀ (anhydrous)

Structural formula



Formula weight

648.56 (anhydrous)

Assay

Not less than 98% on the anhydrous basis

DESCRIPTION

Virtually odourless, white or almost white powder

FUNCTIONAL USES

Carrier

CHARACTERISTICS

IDENTIFICATION

<u>Melting range</u> (Vol.4)	Decomposes above 300°
<u>Solubility</u> (Vol. 4)	Freely soluble in water
<u>Anthrone reaction</u>	Add 5 ml of Anthrone TS (Vol. 4) to 2 ml of a 0.1% aqueous solution of the test sample. React at 80° for 15 min. A deep blue colour develops.
<u>Specific rotation</u> (Vol.4)	$[\alpha]_D^{20}$ between +240° and +248° (10% solution)

PURITY

<u>Water</u> (Vol. 4)	Not more than 15.0% (Karl Fischer Method)
<u>Total ash</u> (Vol. 4)	Not more than 0.1% (500°, 5h)
<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").

METHOD OF ASSAY Determine by HPLC (Vol. 4) using the following conditions:
NOTE: Use deionized water

Sample solution

Weigh accurately about 500 mg of test sample into a 50-ml volumetric flask and add about 40 ml of water. Dissolve the sample completely and dilute to the mark with water.

Standard solution

Dissolve accurately weighed cyclotetraglucose standard (available under the name of cyclotetraose from Hayashibara Co., Ltd, 2-3 Shimoishii 1-chome, Okayama 700, Japan) in water to obtain a solution of about 10 mg/ ml.

Chromatography

Liquid chromatograph equipped with a column oven and a refractive index detector.

Column and packing: strong acidic cation exchange resin

- length: 200 – 400 mm
- diameter: 8 – 10 mm
- temperature: 80°

Mobile phase: water

Flow rate: Adjust to obtain a retention time of 55 – 65 min

Injection volume: 20 µl

The retention time of cyclotetraglucose is approx. 62 min.

System suitability

Upon chromatography of a solution containing about 0.4% cyclotetraglucose and 0.4% glucose, the resolution (Vol. 4) is not less than 1.0 between glucose (first peak) and cyclotetraglucose (second

peak).

Procedure

Inject the sample solution into the chromatograph, and measure the area of the cyclotetraglucose peak. Repeat for the standard solution. Calculate the percentage of cyclotetraglucose in the test sample as follows:

$$\% \text{ cyclotetraglucose (anhydrous basis)} = 100 \times (A_S/A_R)(W_R/W_S)$$

where

A_S and A_R are the areas of the peaks due to cyclotetraglucose for the sample solution and standard solution, respectively; and W_S and W_R are the weights (mg) of the test sample and cyclotetraglucose standard, respectively, corrected for water content.