

# CYCLOTETRAGLUKOSE SYRUP

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**SYNONYMS** Cyclotetraglucose syrup; Cyclic nigerosyl-(1→6)-nigerose syrup; cycloalternan syrup; cycloalternanotetraose syrup; INS No. 1504(ii)

**DEFINITION** A mixture consisting of mono-, di- and oligosaccharides, of which cyclotetraglucose is the major component. It is produced from hydrolyzed food-grade starch by the actions of a mixture of 6- $\alpha$ -glucosyltransferase  $\alpha$ -isomaltosyltransferase derived from *Sporosarcina globispora*, and cyclodextrin glucosyltransferase derived from *Bacillus stearothermophilus*. The final product is a syrup or a spray-dried solid.

**Assay** Not less than 97.0 % of total saccharides and 30 – 40% of cyclotetraglucose on the anhydrous basis

**DESCRIPTION** Colourless and odourless, clear viscous liquid or dry white crystalline mass.

**FUNCTIONAL USES** Carrier

## CHARACTERISTICS

### IDENTIFICATION

Chromatography The retention time of cyclotetraglucose is approx. 62 min using the conditions described under the Method of Assay.

### PURITY

Water (Vol. 4) Not more than 30% for the syrup and not more than 10% for the syrup solids (Karl Fischer Method).

Total ash (Vol. 4) Not more than 0.05% on the anhydrous basis (500°, 5h)

Lead (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”).

Microbiological criteria (Vol.4) Total (Aerobic) plate count: Not more than 300 CFU/g  
Coliforms: Negative in 10 g  
Yeast and moulds: Not more than 100 CFU/g

## METHOD OF ASSAY 1. Total saccharides

Determine by the Anthrone-sulfuric acid method described below.

### Anthrone solution

Add 0.2 g of Anthrone to 100 ml of diluted sulfuric acid (prepare by cautiously adding 75 ml of sulfuric acid to 20 ml of water and make up 100 ml with water). Mix and dissolve completely by ultrasonic treatment.

### Sample solution

Weigh accurately about 1.0 g of the anhydrous basis sample into a 100-ml volumetric flask, and dissolve with water to make 100 ml. Dilute 1 ml of this solution with water to make 100-ml solution accurately.

### Standard solution

Weigh accurately about 1.0 g of glucose (analytical reagent grade, dry solid basis) into a 100-ml volumetric flask, and dissolve with water to make 100 ml. Accurately measure 1 ml of this solution and make 100-ml solution with water.

### Procedure

Add 5 ml of Anthrone solution into each of three test tube (18 mm i.d. × 180 mm) and cool in ice-cold water. Add 0.5 ml of water, standard solution or sample solution into separate tube slowly. After mixing gradually without generating heat, put a glass ball on the tube and heat in boiling water for 10 minutes accurately. After cooling with running water, measure the absorbances ( $A_B$ ,  $A_S$  and  $A_T$  for water blank, standard solution and sample solution, respectively) with the wave length of 620 nm using a 10-mm length cuvette. Calculate the concentration of total saccharides in the sample as follows:

$$\text{Total saccharides (\%)} = (A_T - A_B) / (A_S - A_B) \times W_S / W_T \times 0.908 \times 100$$

where

$W_S$  is the weight of glucose (g, dry solid basis);

$W_T$  is the weight of sample (g, anhydrous basis); and  
0.908 is the anthrone correction factor.

## 2. Cyclotetraglucose

Determine by HPLC (Vol. 4) using the following conditions:

### Sample solution

Weigh accurately about 1.0 g of the anhydrous basis sample into a 50-ml volumetric flask and add about 40 ml of water. Dissolve the sample completely and dilute to 50 ml 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 having known concentration of about 10 mg of cyclotetraglucose per 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

#### Procedure

Inject standard and sample solutions, and measure the areas of the cyclotetraglucose peak. The retention time of cyclotetraglucose is approx. 62 min.

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 of the test sample and standard cyclotetraglucose, respectively (mg, anhydrous basis).