

β-CYCLODEXTRIN

Prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-5 mg/kg bw was established at the 44th JECFA (1995)

SYNONYMS

Beta-cyclodextrin, βCD, BCD, β-Schardinger dextrin, cyclodextrin B, INS No. 459

DEFINITION

A non-reducing cyclic saccharide consisting of seven alpha-1,4-linked D-glucopyranosyl units manufactured by the action of cyclodextrin transglycolase on hydrolysed starch followed by purification of the β-cyclodextrin; purification is by preparation of a β-cyclodextrin/solvent inclusion compound followed by steam-stripping of the solvent before final purification.

Chemical names

Cycloheptaamylose

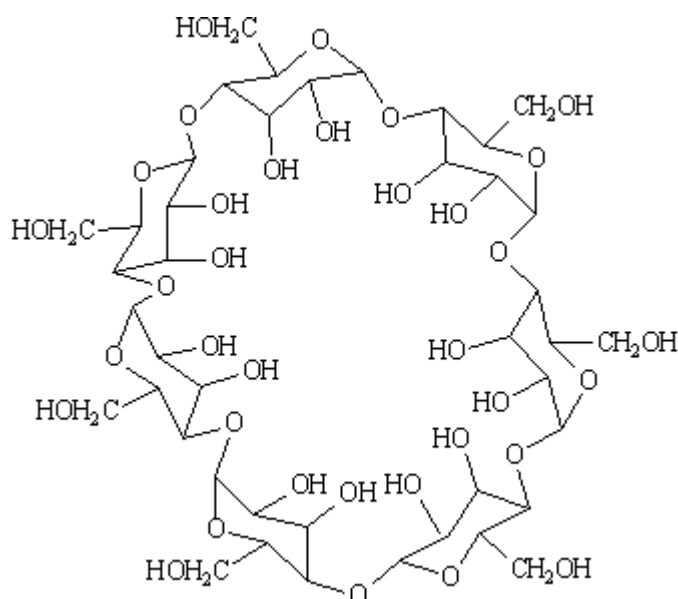
C.A.S. number

7585-39-9

Chemical formula

$(C_6H_{10}O_5)_7$

Structural formula



Formula weight

1135.00

Assay

Not less than 98.0% of $(C_6H_{10}O_5)_7$ on an anhydrous basis

DESCRIPTION

Virtually odourless, slightly sweet tasting white or almost white crystalline solid

FUNCTIONAL USES Encapsulation agent for food additives, flavouring and vitamins

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Sparsingly soluble in water; freely soluble in hot water; slightly soluble in ethanol
<u>Specific rotation</u> (Vol. 4)	[alpha] 25, D: Between +160 and +164° (1% solution)
<u>Infrared absorption</u>	The infrared spectrum of the sample corresponds with that of a reference standard.
<u>Chromatography</u>	The retention time for the major peak in the liquid chromatogram of the sample solution corresponds to that for β-cyclodextrin in the chromatograms of the standard solutions prepared as directed in the Method of Assay.

PURITY

<u>Water</u> (Vol. 4)	Not more than 14% (Karl Fischer Method)
<u>Other cyclodextrins</u>	Not more than 2% on an anhydrous basis See description under TESTS
<u>Residual solvents</u>	Not more than 1 mg/kg of each of toluene and trichloroethylene See description under TESTS
<u>Reducing substances</u> (Vol. 4)	Not more than 1% (as glucose)
<u>Sulfated ash</u> (Vol. 4)	Not more than 0.1%
<u>Lead</u> (Vol. 4)	Not more than 1 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 method described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

<u>Other cyclodextrins</u>	Analyses of alpha- and gamma-cyclodextrins are included in the Method of Assay. Adjust the attenuation of the instrument or adjust sample size to obtain a chromatogram in which the β-cyclodextrin peak height nearly reaches the top of the recording chart. Measure peak heights or peak areas of the alpha-, β- and gamma-cyclodextrin responses. Calculate % other cyclodextrins (CX) using the formula: $\% \text{ Other CX} = \frac{\alpha\text{peaks} + \gamma\text{peaks}}{\alpha\text{peaks} + \beta\text{peaks} + \gamma\text{peaks}} \times 100$
<u>Residual solvents</u>	A dynamic-headspace <i>gas chromatographic technique</i> is used for the following procedure. The organic volatile impurities are trapped on an absorbent trap and the purge gas is vented. The trapped organic volatile

impurities are desorbed from the trap by heating the trap, and carried into the gas chromatograph by back flushing the trap with the carrier gas. Quantitate each solvent by the technique of standard additions.

Purge and Trap Apparatus

(The apparatus is based on that described in the US Environmental Protection Agency Test Method for Purgeable Halocarbons - Method 601): The apparatus consists of three separate sections: the sample purge; the trap; and the desorber. The sample purge is designed to accept 5 ml samples with a water column at least 3 cm deep. The gaseous headspace between the water column and the trap has a total volume of less than 15 ml. The purge gas is passed through the water column as finely-divided bubbles with a diameter of less than 3 mm at the origin. The purge gas is introduced not more than 5 mm from the base of the water column. The trap is not less than 25 cm long and has an inside diameter of not less than 2.67 mm. The trap is packed to contain the indicated minimum lengths of adsorbents in the following order, beginning at the trap inlet:

7.7 cm of 2,6-diphenylene oxide polymer (the 2,6-diphenylene oxide polymer is commercially available as TENAX TA), 7.7 cm of silica gel, and 7.7 cm of coconut charcoal.

The desorber is capable of rapidly heating the trap at 2500. The trap should not be heated higher than 2500.

Condition the assembled trap, prior to initial use, at 2250 overnight with an inert gas at a flow rate of not less than 20 ml per min. Prior to use daily, condition the trap for 15 min at 225°.

Standard Solution

Accurately weigh 50 mg of trichloroethylene and 50 mg of toluene in a 50 ml volumetric flask. Dilute with methanol.

Calibration Solutions

Into five 50 ml volumetric flasks, accurately add 0.5, 1.0, 2.0, 3.0, and 5.0 ml of the Standard Solution and dilute with water. These calibration solutions correspond to the concentrations 10.2, 20.4, 40.8, 61.2 and 102 ng per for each solvent.

Chromatographic system

The purge and trap apparatus is connected to a gas chromatograph with a flame-ionisation detector.

Column: capillary column, 30 m, 0.32 mm diameter, 1 micron film thickness of dimethylpolysiloxane oil (such as DB-1, OV-1).

Temperature programme: 40° for 3 min, then raise to 220° at 40 per min.

Detector: 280°

Carrier gas: Helium

Purge gas: Nitrogen

Flow rate: 40 ml/min

Calibration

Introduce precisely 20 µl of each calibration solution on the wall (inner side)

of the sample purge. Desorb according to equipment instructions. Record the peak areas. Prepare calibration graphs of peak areas versus weight of each solvent introduced into the purge.

Procedure

Introduce on the fritted sparger of the sample purge an accurately weighed amount of sample (W), about 250 mg. Purge and desorb according to equipment instructions. Record the peak area of each solvent and read the corresponding weight (X) from the respective calibration curve.

Calculation

Calculate the amount of each residual solvent by the formula:

$$\text{Residual solvent (mg / kg)} = \frac{X \text{ (ng)}}{W \text{ (mg)}}$$

METHOD OF ASSAY

Principle

β -Cyclodextrin is identified by *liquid chromatography* and quantified by comparison to reference standards containing standard cyclodextrins.

Preparation of sample solution

Weigh accurately about 500 mg of sample. Add 50 ml of twice-distilled water. Heat and stir until the sample has completely dissolved. Cool, adjust the total volume to 100 ml. Filter on a Millex HA 0.45 μ m filter.

Preparation of standard solutions

Use USP grade alpha- and β -cyclodextrin. Samples of gamma-cyclodextrin can be obtained from commercial suppliers such as Aldrich Chemical Co. or Sigma Chemical Co. Prepare three standard solutions (S₁, S₂ and S₃) containing increasing amounts (mg/kg) of alpha-cyclodextrin, β -cyclodextrin and gamma-cyclodextrin as follows:

S₁: 2.0 mg/kg A + 3.0 mg/kg B + 2.0 mg/kg G

S₂: 3.5 mg/kg A + 5.0 mg/kg B + 3.5 mg/kg G

S₃: 5.0 mg/kg A + 8.0 mg/kg B + 5.0 mg/kg G

where

A = alpha- cyclodextrin

B = β -cyclodextrin

G = gamma-cyclodextrin

Apparatus

Liquid chromatograph maintained at a constant temperature of 25° and equipped with a refractive index detector.

Conditions

Column

- length: 25 cm

- diameter: 4.6 mm

- packing: 5 μ m octadecylsilane bonded to silica (Silica C18) with a guard column containing the same packing

Solvent: Water: methanol (94:6)

Flow rate: 0.7 ml/min

Procedure

Inject 10 µl of each of the 3 standard solutions. For each cyclodextrin draw a graph by plotting on the x axis the concentration in g/l and on the y axis the areas of the peaks. Inject 10 µl of the sample solution and determine the area of the eluted β-cyclodextrin peak. The concentration of β-cyclodextrin (L g/l) in the sample solution is then read from the graphs.

Calculation

Calculate the content of β-cyclodextrin in the sample using the formula:

$$B = \frac{L}{C} \times 100$$

where

B = percentage of β-cyclodextrin in the sample

L = the concentration of β-cyclodextrin in the sample solution as determined under "Procedure"

C = the concentration of sample in the sample solution in g/l.