Standard solution: 7.5 µg/mL 1-decanol in *Internal standard solution:* diluted from *Standard stock solution*

Sample solution: Dissolve 750 mg of sample and 50 mg of glucoamylase[3] (EC 3.2.1.3) in 7 mL of *Tris buffer solution.* Add 100 µL of *Internal standard solution* and 50 µL of cyclodextrin glucosyltransferase preparation (500 U/mL).[4] Close tightly, mix, and incubate in a shaking water bath at 40° for 4 h.

Condition a C18 solid-phase extraction column[5] by washing with methanol (2 × 10 mL) and water (4 × 10 mL). Quantitatively transfer the digested sample solution to the conditioned column, and slowly pass it through the column. Wash the column with water (2 × 10 mL). Gently pass nitrogen through the column to dry it (10 min). Apply 2.5 mL of tetrahydrofuran to the column, let stand for 5 min, and collect the eluate. Use the eluate as the *Sample solution.*

Chromatographic system, Appendix IIA

Mode: Gas chromatography

Detector: Flame ionization

Column: 25-m × 0.32-mm capillary column coated with a 0.5-µm layer of dimethylpolysiloxane gum[6]

Column temperature: 60° for 1 min, 20°/min to 300°, 300° for 7 min

Injection port temperature: 265°

Carrier gas: Helium

Flow rate: 1 mL/min

Injection syringe: Heated, gas-tight

Injection volume: 1 µL

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the concentration (mg/kg) of 1-decanol in the sample taken using the following formula:

$$\text{Result} = (R_U/R_S) \times C_S \times (2.5/S) \times 1000$$

R_U = internal standard ratio (1-decanol peak response/1-octanol response) from the *Sample solution*

R_S = internal standard ratio (1-decanol peak response/1-octanol response) from the *Standard solution*

C_S = concentration of 1-decanol in the *Standard solution* (µg/mL)

2.5 = dilution factor for the *Sample solution* from the mL of tetrahydrofuran applied to the solid-phase extraction column

S = mg of sample taken to prepare the *Sample solution*

1000 = µg/mg to mg/kg conversion factor

Acceptance criteria: NMT 20 mg/kg

[3]Gluczyme 8000 (Wacker Chemie, Munich, Germany).
[4]Available from Wacker Chimie (Munich, Germany).
[5]Isolute C18, 10 mL (ICT, Bad Homburg, Germany), or equivalent.
[6]HP-1 (Agilent Technologies), or equivalent.

SPECIFIC TESTS

- **Melting Range or Temperature,** Appendix IIB
  Acceptance criteria: Decomposes above 278°
- **Residue on Ignition (Sulfated Ash),** Appendix IIC
  Sample: 1 to 2 g
  Acceptance criteria: NMT 0.1%
- **Optical (Specific) Rotation,** Appendix IIB
  Sample solution: 10 mg/mL
  Acceptance criteria: $[\alpha]_D^{25}$ between +145° and +151°
- **Water,** *Water Determination, Method I,* Appendix IIB
  Acceptance criteria: NMT 11%

beta-Cyclodextrin

First Published: Prior to FCC 6

β-Cyclodextrin
BCD

$(C_6H_{10}O_5)_7$ Formula Wt 1135.0

INS: 459 CAS: [7585-39-9]

DESCRIPTION

Beta-Cyclodextrin occurs as a white, fine, crystalline solid, frequently a fine, crystalline powder. It is a nonreducing cyclic compound consisting of seven alpha-(1,4) linked D-glucopyranosyl units. It is slightly soluble in water.

Function: Encapsulating agent; stabilizer

Packaging and Storage: Store in tight containers in a dry place.

IDENTIFICATION

- **Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP beta-Cyclodextrin RS
  Sample and Standard preparation: *K*
  Analysis: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*
- **Chromatography,** Appendix IIA
  Analysis: The retention time of the major peak in the chromatogram of *Sample solution* corresponds to that in the chromatogram of *Standard solution,* obtained as directed in the *Assay* (below).

ASSAY

- **Procedure**
  Mobile phase: Acetonitrile and water [65:35], filtered and degassed
  Internal standard solution: 20 mg/mL of glycerol, filtered through a 0.45-µm membrane filter [Note—Use fresh or store in a freezer and thaw in hot water.]
  Standard stock solution: 10 mg/mL of USP beta-Cyclodextrin RS [Note—Use fresh or store in a freezer and thaw in hot water.]
  Standard solution: Mix 1.0 mL of the *Standard stock solution* with 1.0 mL of *Internal standard solution.*

System suitability solution: 5 mg/mL each of USP alpha-Cyclodextrin RS and USP beta-Cyclodextrin RS, filtered through a 0.45-µm membrane filter

Sample: 1 g

Sample stock solution: Transfer the *Sample* into a 100-mL volumetric flask, dilute to volume with water, and mix. Filter this solution through a 0.45-µm membrane filter.

Sample solution: Mix 1.0 mL of the filtered *Sample stock solution* with 1.0 mL of *Internal standard solution.*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index

Column: 25-cm × 4.6-mm (id) column packed with 10-µm porous silica gel bonded with aminopropylsilane (Alltech 35643, or equivalent), and a guard column that contains the same packing

Column temperature: 25° ± 2°

Detector temperature: 25°

Flow rate: About 2.0 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirement 1: The relative standard deviation for replicate injections is NMT 2.0%.

Suitability requirement 2: The alpha-cyclodextrin and beta-cyclodextrin peaks exhibit baseline separation. [NOTE—The relative retention times for alpha-cyclodextrin and beta-cyclodextrin are about 0.8 and 1.0, respectively.]

Analysis: Separately inject the *Sample solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_6H_{10}O_5)_7$ in the portion of the *Sample* taken by the formula:

$$\text{Result} = C \times (R_U / R_S) \times 100$$

C = concentration (mg/mL) of anhydrous beta-cyclodextrin in the *Standard solution,* corrected for moisture content by a titrimetric water determination

R_U = peak response ratio of the beta-cyclodextrin peak to the internal standard peak obtained from the *Sample solution*

R_S = peak response ratio of the beta-cyclodextrin peak to the internal standard peak obtained from the *Standard solution*

Acceptance criteria: NLT 98.0% and NMT 101.0% of $(C_6H_{10}O_5)_7$ as beta-cyclodextrin, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **LEAD,** *Lead Limit Test, Flame Absorption Spectrophotometric Method,* Appendix IIIB

Sample: 10 g

Acceptance criteria: NMT 1 mg/kg

Organic Impurities

- **REDUCING SUGARS (DEXTROSE EQUIVALENT),** *Reducing Sugars,* Appendix X

Sample: 60 to 120 mg

Acceptance criteria: NMT 1.0%, calculated on the anhydrous basis

- **TOLUENE**

Toluene standard stock solution: 1000 µg/mL (or 1.153 µL/mL) of toluene in methanol

Toluene standard solutions: 10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL of toluene in methanol: from *Toluene standard stock solution*

Trifluorotoluene standard stock solution: 800 µg/mL (or 0.673 µL/mL) of α,α,α-trifluorotoluene in methanol

Trifluorotoluene standard solutions: 80 µg/mL, 400 µg /mL, and 800 µg/mL of α,α,α-trifluorotoluene in methanol, from *Trifluorotoluene standard stock solution* [NOTE—These are the surrogate standards.]

Sample: 500 mg

Chromatographic system, Appendix IIA

Mode: Gas chromatography connected to a purge and trap apparatus (below)

Detector: Photoionization

Column: 30-m × 0.53-mm (id) fused silica open tubular column, or equivalent, with a 1.5-µm crossbonded 5% diphenyl, 95% dimethyl polysiloxane (Restek RTX-5, or equivalent) stationary phase

Column temperature: Hold at 40° for 2 min, increase at 20°/min to 180°, hold at 180° for 2 min.

Flow rate: 20 mL/min

Carrier gas: Helium (ultra-high-purity)

Purge and trap apparatus: The purging apparatus uses disposable 15- × 150-mm test tubes. Use ultra-high-purity helium to purge the sample for 15 min at a flow rate of 60 mL/min. Maintain at 100° all lines that the sample vapor passes through in the purge module. The trap consists of a 30.5-cm × 2.7-mm (id) stainless steel tube, with a packing of a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide (Tenax, or equivalent). The length of the packing in the tube is 24 cm. The entire void volume of the trap is at the vented end of the trap column. Maintain the trap at 100°. Recondition the trap for a subsequent run by baking it for 5 min at 190°.

Calibration: Place an empty purge tube into the purging apparatus. Fill a 5-mL syringe with 0.5 N sodium hydroxide, and inject into this solution an accurately measured 5-µL aliquot of 10 µg/mL of *Toluene standard solution.* Introduce this solution into the purge tube. Start the instrument for the run, automated if desired, by purging for 15 min, then heating the trap at 180° for 3 min. Repeat this sequence for each of the *Toluene standard solutions* and *Trifluorotoluene standard solutions.* Plot standard curves of the standard concentration (C_S), in µg/mL, versus detector response (r_S) for the *Toluene standard solutions* and *Trifluorotoluene standard solutions.*

Analysis: Place the *Sample* in a purge tube with 0.1 g of salicylic acid. Attach the purge tube to the purging

apparatus. Add 5 µL of 400 µg/mL of *Trifluorotoluene standard solution* to a 5-mL syringe filled with 0.5 N sodium hydroxide. Add the contents of the syringe to the sample/salicylic acid mixture in the purge apparatus, and start the run, automated if desired, by purging for 15 min, then heating the trap at 180° for 3 min. The procedure is valid only when the detector response of the surrogate standard (*Trifluorotoluene standard solution*) in the sample preparation is within ±15% of the value from the standard curve for *Trifluorotoluene standard*. Calculate the concentration, in µg/g (numerically equivalent to mg/kg), of toluene in the *Sample* taken by the formula:

$$\text{Result} = 5C_S / W_S$$

C_S = concentration of toluene (µg/mL) in the sample, calculated from the standard curve for the *Toluene standard solutions*

W_S = weight of *Sample* (g) taken

Acceptance criteria: NMT 1 mg/kg

- **TRICHLOROETHYLENE**

Standard stock solution: 1 mg/mL of reagent-grade trichloroethylene in methanol

Standard solutions: Transfer 0.5, 1.0, 2.0, 3.0, and 5.0 mL of the *Standard stock solution* into five 50-mL volumetric flasks and dilute to volume with water. These *Standard solutions* contain 10 ng/µL, 20 ng/µL, 40 ng/µL, 60 ng/µL, and 100 ng/µL of trichloroethylene.

Sample: 250 mg

Chromatographic system, Appendix IIA

Mode: Gas chromatography

Detector: Flame-ionization

Column: 30-m × 0.32-mm (id) capillary column coated with a 1-µm film thickness of dimethylpolysiloxane oil (such as DB-1, OV-1, or equivalent)

Column temperature: Hold at 40° for 3 min, increase at 4°/min to 220°.

Detector temperature: 280°

Carrier gas: Helium

Purge gas: Nitrogen

Flow rate: 40 mL/min

Injection size: 20 µL

Purge and trap apparatus: The apparatus[1] comprises three sections: the sample purge, the trap, and the desorber. The sample purge accepts 5-mL samples with a water column NLT 3 cm deep, and the gaseous headspace between the water column and the trap has a total volume of NMT 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 and is introduced NMT 5 mm from the base of the water column. Use a trap not shorter than or narrower than 25 cm × 2.67 mm (id). Pack the trap 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 (TENAX GC, or equivalent), 7.7 cm of silica gel, and 7.7 cm of coconut charcoal. The desorber is capable of rapidly heating the trap to 250°, which is the maximum temperature to be used. Condition the assembled trap before use at 225° overnight with an inert gas at a flow rate of not less than 20 mL/min. Before daily use, condition the trap for 15 min at 225°.

Analysis: Introduce exactly 20 µL of each *Standard solution* on the inner wall of the sample purge. Desorb according to equipment instructions, and record the peak areas. Prepare a calibration curve by plotting the peak area responses versus the weight of trichloroethylene introduced into the purge. Introduce the *Sample* on the fritted sparger of the sample purge. Purge and desorb according to equipment instructions. Record the peak area of trichloroethylene, and read the corresponding weight (*X*) of trichloroethylene from the calibration curve. Calculate the amount of trichloroethylene, in mg/kg, by the formula:

$$\text{Result} = X/W$$

X = weight of trichloroethylene (ng) in the sample, determined from the calibration curve

W = weight of *Sample* (mg) taken

Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS

- **OPTICAL (SPECIFIC) ROTATION,** Appendix IIB

Sample solution: 1 g in 100 mL of water

Acceptance criteria: $[\alpha]_D^{20}$ between +160° and +164°, calculated on the anhydrous basis

- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC

Sample: 1 to 2 g

Acceptance criteria: NMT 0.1%

- **WATER,** *Water Determination,* Appendix IIB

Acceptance criteria: NMT 14.0%

[1]The apparatus used is based on that described in the U.S. Environmental Protection Agency Test Method for Purgeable Halocarbons—Method 601.