Dissolution rates of Digitoxin Tablets after 30 minutes and 60 minutes should be not less than 60% and 85%, respectively.

No retest requirement is applied to Digitoxin Tablets.

Dissolution rate (%) with respect to the labeled amount of digitoxin ($C_{41}H_{64}O_{13}$) for 30 minutes

$$= W_S \times \frac{F_{30} - F_B}{F_S - F_B} \times \frac{1}{C}$$

Dissolution rate (%) with respect to the labeled amount of digitoxin ($C_{41}H_{64}O_{13}$) for 60 minutes

$$= W_S \times \left(\frac{F_{60} - F_B}{F_S - F_B} + \frac{F_{30} - F_B}{F_S - F_B} \times \frac{a + 15}{500} \right) \times \frac{1}{C}$$

W_S: Amount (mg) of Digitoxin Reference Standard.

C: The labeled amount (mg) of digitoxin ($C_{14}H_{64}O_{13}$) in 1 tablet.

a + 15: Measured volume (mL) of dissolved solution at the specified time.

Content uniformity Transfer 1 tablet of Digitoxin Tablets to a 50-mL beaker, add 0.5 mL of water to disintegrate the tablet, add 5 mL of acetonitrile, and warm on a water bath for 5 minutes, covering the beaker with a watch glass. After cooling, transfer the solution to separator A, rinse the beaker with 30 mL of chloroform and then with 20 mL of water, transfer the rinsings to separator A, and extract by vigorous shaking. Transfer the chloroform extract to separator B containing 5 mL of a solution of sodium hydrogen carbonate (1 in 100), and shake to wash. Filter the chloroform layer through a pledget of absorbent cotton, previously moistened with chloroform. Extract the water layer in separator A with two 30-mL portions of chloroform, wash the chloroform extract with a solution of sodium hydrogen carbonate (1 in 100) in separator B, filter in the same manner, and combine the filtrate with the first one. Evaporate this filtrate to dryness under reduced pressure by warming, add diluted ethanol (95) (4 in 5) to make exactly V mL of a solution containing 5 μg of digitoxin ($C_{41}H_{64}O_{13}$) per ml. Shake vigorously for 20 minutes to dissolve, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Digitoxin Reference Standard, previously dried at 100°C for 2 hours, and dissolve in diluted ethanol (95) (4 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted ethanol (95) (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and diluted ethanol (95) (4 in 5) into brown glass-stoppered test tubes T, S and B. Add exactly 10 mL each of 0.02 w/v% L-ascorbic acid-hydrochloric acid TS, shake well, and immediately add exactly 1 mL each of dilute hydrogen peroxide TS. Shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_T, F_S and F_B, of these solutions at 400 nm of the excitation wavelength and at about 570 nm of the fluorescence wavelength as directed under the Fluorometry, respectively.

Amount (mg) of digitoxin ($C_{41}H_{64}O_{13}$)

$$= \text{amount (mg) of Digitoxin Reference Standard} \times \frac{F_T - F_B}{F_S - F_B} \times \frac{V}{2000}$$

Assay Weigh accurately and powder not less than 20 Digitoxin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of digitoxin ($C_{41}H_{64}O_{13}$), and shake with 12.5 mL of water for 10 minutes. Add exactly 10 mL of the internal standard solution, shake for 20 minutes, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.02 g of Digitoxin Reference Standard, previously dried in vacuum at 100°C for 2 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of the solution, add exactly 10 mL of the internal standard solution, add 12.5 mL of water, then methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Digitoxin.

Amount (mg) of digitoxin ($C_{41}H_{64}O_{13}$)

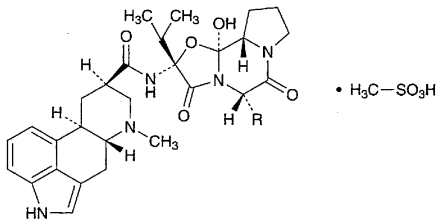
$$= \text{amount (mg) of Digitoxin Reference Standard} \times \frac{Q_T}{Q_S} \times 0.025$$

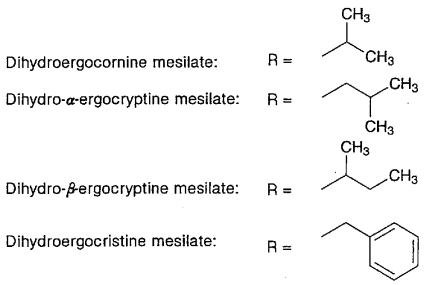
Internal standard solution—A solution of acenaphthene in methanol (3 in 1,000,000).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Dihydroergotoxine Mesilate

メシル酸ジヒドロエルゴトキシン





Dihydroergocornine mesilate
$C_{31}H_{41}N_5O_5.CH_4O_3S$: 659.79
(5′*S*,10*R*)-9,10-Dihydro-12′-hydroxy-2′,5′-diisopropylergotaman-3′,6′,18-trione monomethanesulfonate

Dihydro-α-ergocryptine mesilate
$C_{32}H_{43}N_5O_5.CH_4O_3S$: 673.82
(5′*S*,10*R*)-9,10-Dihydro-12′-hydroxy-2′-isopropyl-5′-(2-methylpropyl)ergotaman-3′,6′,18-trione monomethanesulfonate

Dihydro-β-ergocryptine mesilate
$C_{32}H_{43}N_5O_5.CH_4O_3S$: 673.82
(5′*S*,10*R*)-9,10-Dihydro-12′-hydroxy-2′-isopropyl-5′-(1-methylpropyl)ergotaman-3′,6′,18-trione monomethanesulfonate

Dihydroergocristine mesilate
$C_{35}H_{41}N_5O_5.CH_4O_3S$: 707.84

(5′*S*,10*R*)-5′-Benzyl-9,10-dihydro-12′-hydroxy-2′-isopropylergotaman-3′,6′,18-trione monomethanesulfonate
[*8067-24-1*, Dihydroergotoxine Mesilate]

Dihydroergotoxine Mesilate contains not less than 97.0% and not more than 103.0% of dihydroergotoxine mesilate [as a mixture of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5.CH_4O_3S$), dihydro-α-ergocryptine mesilate ($C_{32}H_{43}N_5O_5.CH_4O_3S$), dihydro-β-ergocryptine mesilate ($C_{32}H_{43}N_5O_5.CH_4O_3S$) and dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5.CH_4O_3S$)], calculated on the anhydrous basis. The relative contents of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5.CH_4O_3S$), dihydroergocryptine mesilate ($C_{32}H_{43}N_5O_5.CH_4O_3S$) and dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5.CH_4O_3S$) are 30.3–36.3% each, and the content ratio of dihydro-α-ergocryptine mesilate and dihydro-β-ergocryptine mesilate is 1.5–2.5:1.

Description Dihydroergotoxine Mesilate occurs as a white to pale yellow powder.

It is soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, in acetonitrile and in chloroform, and practically insoluble in diethyl ether.

Identification Determine the infrared absorption spectrum of Dihydroergotoxine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +11.0 – +15.0° (0.2 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Dihydroergotoxine Mesilate in 20 mL of water: the solution is clear and the color of the solution is not more intense than that of the following control solution.

Control solution: To a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 0.4 mL of Cupric Sulfate Stock CS and 2.4 mL of Ferric Chloride Stock CS add diluted hydrochloric acid (1 in 40) to make exactly 200 mL.

(2) Heavy metals—Proceed with 1.0 g of Dihydroergotoxine Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately 0.100 g of Dihydroergotoxine Mesilate, dissolve it in a mixture of chloroform and methanol (9:1) to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately 0.010 g of dihydroergocristine mesilate for thin-layer chromatography, and dissolve in a mixture of chloroform and methanol (9:1) to make exactly 100 mL. Pipet 6 mL, 4 mL and 2 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 10 mL, respectively, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography without putting the filter paper in the developing vessel. Spot 5 μL each of the sample solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate with the aid of a cool air stream. Immediately after that, develop the plate again with a newly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate within 1 minute with the aid of a cool air stream. Spray evenly *p*-dimethylaminobenzal-dehyde-hydrochloric acid TS on the plate, dry the plate within 2 minutes with the aid of a cool air stream, and heat it at 40°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), not more than 2 spots are more intense than that from the standard solution (2), and not more than 4 spots are more intense than that from the standard solution (3).

Water Not more than 5.0% (0.2 g, direct titration).

Residue on ignition Not more than 0.10% (1 g).

Assay **(1)** Dihydroergotoxine mesilate—Weigh accurately about 0.03 g each of Dihydroergotoxine Mesilate and Dihydroergotoxine Mesilate Reference Standard, and dissolve them separately in a suitable amount of a mixture of water and acetonitrile (3:1). To these solutions add exactly 10 mL of the internal standard solution and an amount of a mixture of water and acetonitrile (3:1) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following operating conditions, and calculate the ratios of the peak areas of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to the peak area of the internal standard of these solutions.

Amount (mg) of dihydroergotoxine mesilate
= amount (mg) of Dihydroergotoxine Mesilate Reference Standard, calculated on the anhydrous basis

$$\times \frac{M_{TA} + M_{TB} + M_{TC} + M_{TD}}{M_{SA} + M_{SB} + M_{SC} + M_{SD}}$$

M_{TA}: Ratio of the peak area of dihydroergocornine to that of the internal standard of the sample solution × 659.80

M_{TB}: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the sample solution × 673.83

M_{TC}: Ratio of the peak area of dihydroergocristine to that of the internal standard of the sample solution × 707.85

M_{TD}: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the sample solution × 673.83

M_{SA}: Ratio of the peak area of dihydroergocornine to that of the internal standard of the standard solution × 659.80

M_{SB}: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the standard solution × 673.83

M_{SC}: Ratio of the peak area of dihydroergocristine to that of the internal standard of the standard solution × 707.85

M_{SD}: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the standard solution × 673.83

Internal standard solution—Dissolve 0.04 g of chloramphenicol in a mixture of water and acetonitrile (3:1) to make 250 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and triethylamine (30:10:1).

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol is about 5 minutes.

Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard, dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine in this order with the resolution between the peaks of dihydro-α-ergocryptine and dihydroergocristine being not less than 1.5.

(2) Relative contents of dihydroergocornine mesilate, dihydroergocryptine mesilate and dihydroergocristine mesilate—Calculate the relative amounts of dihydroergocornine mesilate, dihydroergocryptine mesilate (dihydro-α-ergocryptine mesilate and dihydro-β-ergocryptine mesilate) and dihydroergocristine mesilate from the chromatogram obtained in Assay (1) for the sample solution using the following equations:

$$\text{Relative amount (\%) of dihydroergocornine mesilate} = \frac{M_{TA}}{M_{TA} + M_{TB} + M_{TC} + M_{TD}} \times 100$$

$$\text{Relative amount (\%) of dihydroergocryptine mesilate} = \frac{M_{TB} + M_{TD}}{M_{TA} + M_{TB} + M_{TC} + M_{TD}} \times 100$$

$$\text{Relative amount (\%) of dihydroergocristine mesilate} = \frac{M_{TC}}{M_{TA} + M_{TB} + M_{TC} + M_{TD}} \times 100$$

(3) Ratio of the content of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate—Calculate the ratio of the amount of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate from the chromatogram obtained in Assay (1) for the sample solution using the following equations:

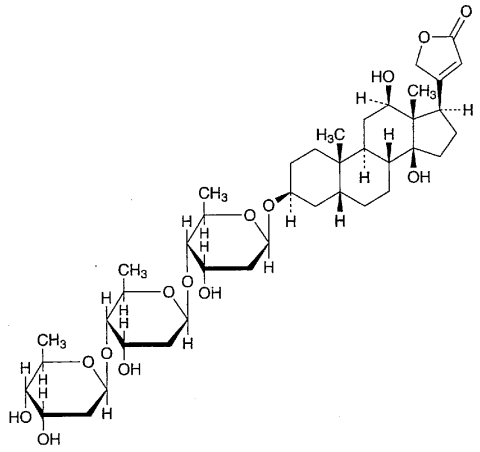
$$\text{Ratio of the content of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate} = \frac{M_{TB}}{M_{TD}}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Digoxin

ジゴキシン



$C_{41}H_{64}O_{14}$: 780.94

3β-[*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide [*20830-75-5*]

Digoxin, when dried, contains not less than 96.0% and not more than 106.0% of $C_{41}H_{64}O_{14}$.

Description Digoxin occurs as colorless to white crystals or white, crystalline powder. It is odorless.

It is freely soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in acetic acid (100), and practically insoluble in water, in chloroform, in diethyl ether and in propylene glycol.

Identification **(1)** Transfer 1 mg of Digoxin to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring free from a reddish color is produced, and the color of the upper layer near the contact zone changes to green through purple. Finally the entire acetic acid layer shows a green color through a deep blue color.

(2) Dissolve 1 mg each of Digoxin and Digoxin Reference Standard in 50 mL of a mixture of chloroform and ethanol (95) (1:1), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 110°C for 10 minutes: the spot from the sample solution shows the same *Rf* value as the spot from the standard solution.

Optical rotation $[\alpha]_{546.1}^{25}$: +13.3 – +14.3° (after drying, 1 g, pyridine, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Digoxin in 15 mL of diluted ethanol (95) (4 in 5) by warming: the solution is clear and colorless.