**Optical rotation** $[\alpha]_D^{20}$: +16 – +18° (after drying. 0.5 g, chloroform, 20 mL, 200 mm).

**Purity** Digitonin—Dissolve 0.010 g of Digitoxin in 2 mL of ethanol (95) in a test tube, having the inner walls which are free from scratches, add 2 mL of a solution of cholesterol in ethanol (95) (1 in 200), mix gently, and allow to stand for 10 minutes: no turbidity is produced.

**Loss on drying** Not more than 1.5% (0.5 g, in vacuum, 100°C, 2 hours).

**Residue on ignition** Not more than 0.5% (0.1 g).

**Assay** Dissolve about 0.02 g each of Digitoxin and Digitoxin Reference Standard, previously dried and accurately weighed, in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution to each solution, add 12.5 mL of water, then add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 50 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of digitoxin to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{41}H_{64}O_{13} = \text{amount (mg) of Digitoxin Reference Standard} \times \frac{Q_T}{Q_S}$$

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

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol and water (3:1).

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

Selection of column: Proceed with 50 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of digitoxin and the internal standard in this order with the resolution between these peaks being not less than 6.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Digitoxin Tablets

ジギトキシン錠

Digitoxin Tablets contain not less than 90% and not more than 110% of the labeled amount of digitoxin ($C_{41}H_{64}O_{13}$: 764.94).

**Method of preparation** Prepare as directed under Tablets, with Digitoxin.

**Identification** **(1)** Place a portion of powdered Digitoxin Tablets, equivalent to 2 mg of digitoxin ($C_{41}H_{64}O_{13}$) according to the labeled amount, in a separator, shake with 30 mL of water, and shake vigorously with 30 mL of chloroform. Filter the chloroform extract with a funnel on which a small amount of anhydrous sodium sulfate is placed, and transfer to a round-bottomed flask connected by a universal joint. Evaporate the solution to dryness by warming under reduced pressure, and dissolve the residue in 10 mL of chloroform. Transfer 5 mL of this solution to a small test tube about 10 mm in inside diameter, and evaporate to dryness on a water bath with the aid of a current of air. Proceed with the residue as directed in the Identification (1) under Digitoxin.

**(2)** Evaporate 4 mL of the chloroform solution obtained in (1) to dryness, by warming under reduced pressure, add a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100) to the residue, and dissolve by shaking. Proceed with 2 mL of this solution as directed in the Identification (2) under Digitoxin.

**Dissolution test** Take 1 tablet of Digitoxin Tablets, and perform the test using 500 mL of diluted hydrochloric acid (3 in 500), degassed by a suitable method, as the test solution at 100 revolutions per minute as directed in the Method 1 under the Dissolution Test. At 30 minutes after starting the test, take $a$ + 15 mL of the dissolved solution, and immediately add the same volume of fresh test solution, previously warmed at 37 ± 0.5°C, to the vessel carefully. Filter $a$ + 15 mL of the dissolved solution through a membrane filter (less than 0.8 μm in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Measure exactly $a$ mL of the sample solution, equivalent to about 2 μg of digitoxin ($C_{41}H_{64}O_{13}$) according to the labeled amount, transfer to a glass-stoppered centrifuge tube $T_{30}$, and warm at 37 ± 0.5°C for 30 minutes. Further, at 60 minutes after starting the test, take $a$ + 15 mL of the dissolved solution, proceed in the same manner, measure exactly $a$ mL of the sample solution, and transfer to a glass-stoppered centrifuge tube $T_{60}$. Separately, weigh accurately 100 times the labeled amount of Digitoxin Reference Standard, previously dried under reduced pressure at 100°C for 2 hours, and dissolve in ethanol (95) to make exactly 100 mL. Measure exactly 1 mL of this solution, add the test solution to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and filter through a membrane filter (less than 0.8 μm in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the standard solution. Measure exactly $a$ mL each of the standard solution and the test solution, transfer to glass-stoppered centrifuge tubes $T_S$ and $T_B$, respectively. Add exactly 7 mL of chloroform to each of the glass-stoppered centrifuge tubes $T_{30}$, $T_{60}$, $T_S$ and $T_B$, shake vigorously for 10 minutes and centrifuge. Discard the aqueous layer, measure exactly 5 mL of the chloroform layer, transfer to brown test tubes $T'_{30}$, $T'_{60}$, $T'_S$ and $T'_B$, evaporate the chloroform, add exactly 4 mL each of 0.05 w/v% L-ascorbic acid-hydrochloric acid TS, shake well, and allow to stand for 10 minutes. Then add exactly 0.5 mL each of dilute hydrogen peroxide TS, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, $F_{30}$, $F_{60}$, $F_S$ and $F_B$, of these solutions at about 395 nm of the excitation wavelength and at about 560 nm of the fluorescence wavelength as directed under the Fluorometry, respectively.

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 $\mu$g 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 ($C_{41}H_{64}O_{13}$)

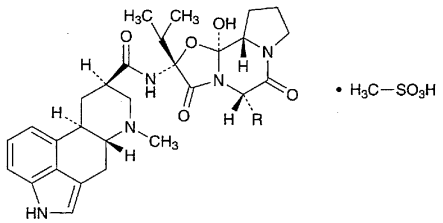
$$= \text{amount (mg) of Digitoxin Reference Standard} \times \frac{Q_T}{Q_S} \times 0.025$$

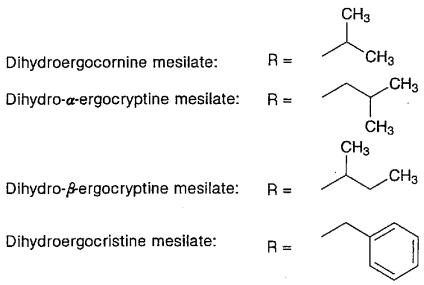
*Internal standard solution*—A solution of acenaphthene in methanol (3 in 1,000,000).

**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