is formed.

(3) To 10 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 15-mL portions of chloroform. Combine the extracts, wash with three 10-mL portions of water, evaporate the chloroform on a water bath, and dry the residue in a desiccator (in vacuum, silica gel, 55°C) for 5 hours: the residue melts between 103°C and 106°C.

(4) A solution of Difenidol Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Difenidol Hydrochloride in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.7 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Difenidol Hydrochloride in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Difenidol Hydrochloride 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) Arsenic—Prepare the test solution with 2.0 g of Difenidol Hydrochloride according to Method 3, and perform the test using Apparatus B (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 g of Difenidol Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 0.010 g of 1,1-diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography in methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, methanol and acetic acid (100) (10:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours).

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

Assay Weigh accurately about 0.35 g of Difenidol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100) by warming if necessary, cool, add 30 mL of acetic anhydride, and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.296 mg of $C_{21}H_{27}NO.HCl$

Containers and storage Containers—Well-closed containers.

Digitoxin

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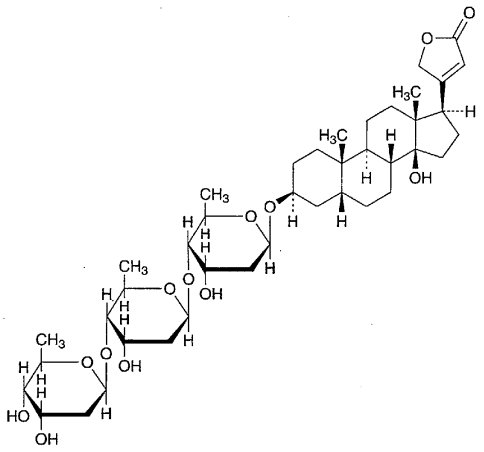

$C_{41}H_{64}O_{13}$: 764.94
3β-[*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-14-hydroxy-5β,14β-card-20(22)-enolide [*71-63-6*]

Digitoxin, when dried, contains not less than 90.0% of $C_{41}H_{64}O_{13}$.

Description Digitoxin occurs as a white to light yellowish white, crystalline powder. It is odorless.

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

Identification **(1)** Transfer 1 mg of Digitoxin 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 color of the entire acetic acid layer changes to green through deep blue.

(2) To 2 mg of Digitoxin add 25 mL of a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100), and dissolve by shaking. Take 2 mL of this solution, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and mix: a red-purple color develops slowly, and then fades.

(3) Dissolve 1 mg each of Digitoxin and Digitoxin Reference Standard in a mixture of chloroform and ethanol (95) (1:1) to make 50 mL, 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 $[\alpha]_D^{20}$: +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

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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.