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

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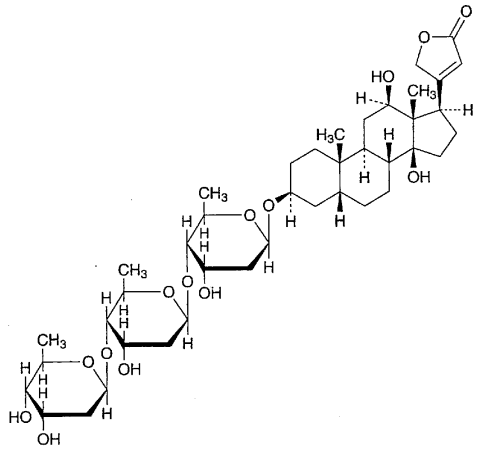

$C_{41}H_{64}O_{14}$: 780.94

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.

(2) Related substances—(i) Gitoxin standard solution: Weigh accurately 10.0 mg of Gitoxin Reference Standard, previously dried at 105°C for 1 hour, and dissolve in a mixture of chloroform and methanol (2:1) to make exactly 100 mL. Pipet 10 mL of this solution and dilute with a mixture of chloroform and methanol (2:1) to make exactly 100 mL. This solution contains 10 μg of Gitoxin Reference Standard per mL. Store the solution in a refrigerator.

(ii) Propylene glycol-hydrochloric acid mixture: Mix equal volumes of propylene glycol and hydrochloric acid. Store the mixture in a refrigerator, and warm to 20°C before use.

(iii) Procedure: Pipet 1 mL of the sample stock solution prepared in the Assay and 1 mL of the gitoxin standard solution into separate 50-mL beakers, T and S, and evaporate on a water bath to dryness with the aid of a current of air, avoiding to overheat. After cooling, add 10 mL each of a mixture of propylene glycol and hydrochloric acid, and place in a water bath at 20°C for 28 minutes swirling the solutions frequently. Determine the fluorescence intensities, F_T and F_S, of each solution at 355 nm of the excitation wavelength and at 465 nm of the fluorescence wavelength exactly 30 minutes after adding a mixture of propylene glycol and hydrochloric acid, as directed under the Fluorometry: F_T is not greater than F_S.

Loss on drying Not more than 1.0% (0.5 g, in vacuum, 105°C, 1 hour).

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

Assay Weigh accurately about 0.025 g each of Digoxin and Digoxin Reference Standard, previously dried in vacuum at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Use these solutions as the sample stock solution and the standard stock solution, respectively. Measure exactly 10 mL each of these solutions, dilute with ethanol (95) to exactly 100 mL, and use the solutions as the sample solution and the standard solution, respectively. Transfer 5 mL each of the sample solution and the standard solution to separate conical flasks, evaporate on a water bath to dryness with the aid of a current of air, and allow to stand in a desiccator (in vacuum, phosphorus (V) oxide) for 15 minutes. Add 5.0 mL each of alkaline 1,3-dinitrobenzene TS, and allow to stand, with frequent swirling, at a temperature not exceeding 30°C for 5 minutes. Perform the test with these solutions, using ethanol (95) as the blank, as directed under the Ultraviolet-visible Spectrophotometry. Determine the maximum absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and the standard solution by repeating the determination at 620 nm at 1-minute intervals, respectively.

$$\text{Amount (mg) of } C_{41}H_{64}O_{14} = \text{amount (mg) of Digoxin Reference Standard} \times \frac{A_T}{A_S}$$

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

Digoxin Injection

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Digoxin Injection is an aqueous solution for injection. It contains not less than 90% and not more than 110% of the labeled amount of digoxin ($C_{41}H_{64}O_{14}$: 780.94).

Method of preparation Prepare as directed under Injections, with a solution of Digoxin in 5 to 50 vol% ethanol.

Description Digoxin Injection is a clear, colorless liquid.

Identification Evaporate 2 mL of the sample solution obtained in the Assay on a water bath to dryness. Cool, and dissolve the residue in 5 mL of alkaline 1,3-dinitrobenzene TS: a blue color develops within 10 minutes, then fades gradually.

Purity Related substances—Proceed as directed in the Purity (2) under Digoxin, using 10 mL of the sample solution obtained in the Assay under Digoxin Injection instead of 1 mL of the sample stock solution obtained in the Assay under Digoxin.

Assay Transfer an exactly measured volume of Digoxin Injection, equivalent to 2.5 mg of digoxin ($C_{41}H_{64}O_{14}$), to a separator, add water to make 50 mL, then add 1 mL of dilute sulfuric acid, and extract with 35-mL, 30-mL and 30-mL portions of a mixture of chloroform and 1-propanol (5:1) successively, wash each extract with the same 5 mL of water, and filter the extracts through absorbent cotton moistened with chloroform into a 100-mL volumetric flask. Combine all the extracts, add ethanol (95) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of Digoxin Reference Standard, previously dried in vacuum at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of the solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and the standard solution into separate conical flasks. Evaporate on a water bath with the aid of a current of air nearly to dryness, and allow to stand in a desiccator (in vacuum, phosphorus (V) oxide) for 15 minutes. Dissolve each residue in 5 mL of acidic iron (III) chloride TS with occasional stirring, allow to stand at a temperature not exceeding 30°C for 10 minutes, protected from light, and filter through a plug of glass wool, if necessary. Perform the test with these solutions, using acidic iron (III) chloride TS as the blank, as directed under the Ultraviolet-visible Spectrophotometry. Detemine the maximum absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and the standard solution by repeating the determination at 590 nm at 2-minute intervals, respectively.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = \text{amount (mg) of Digoxin Reference Standard} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.