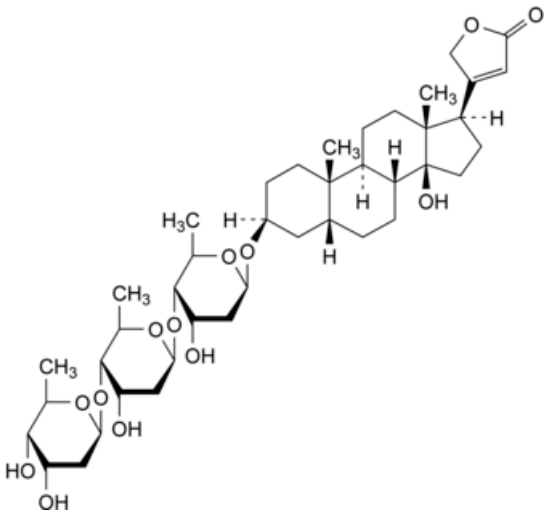
Edition: BP 2025 (Ph. Eur. 11.6 update)

Digitoxin

General Notices

(Ph. Eur. monograph 0078)



$C_{41}H_{64}O_{13}$ 765 *71-63-6*

Action and use

Na/K-ATPase inhibitor; cardiac glycoside.

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DEFINITION

Digitoxin contains not less than 95.0 per cent and not more than the equivalent of 103.0 per cent of 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*-hexopyranosyl)oxy]-14-hydroxy-5β,14β-card-20(22)-enolide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, freely soluble in a mixture of equal volumes of methanol and methylene chloride, slightly soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with *digitoxin CRS*.
B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).
C. Suspend about 0.5 mg in 0.2 mL of *alcohol (60 per cent V/V) R*. Add 0.1 mL of *dinitrobenzoic acid solution R* and 0.1 mL of *dilute sodium hydroxide solution R*. A violet colour develops.
D. Dissolve about 0.5 mg in 1 mL of *glacial acetic acid R*, heating gently, allow to cool and add 0.05 mL of *ferric chloride solution R1*. Cautiously add 1 mL of *sulfuric acid R*, avoiding mixing the two liquids. A brown ring develops at the interface and on standing a green, then blue colour passes to the upper layer.

TESTS

Appearance of solution

Dissolve 50 mg in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 mL with the same mixture of solvents. The solution is clear (2.2.1) and colourless (2.2.2, Method I).

Specific optical rotation (2.2.7)

Dissolve 0.25 g in *chloroform R* and dilute to 10.0 mL with the same solvent. The specific optical rotation is + 16.0 to + 18.5.

Related substances

Examine by thin-layer chromatography (2.2.27), using a *TLC silica gel G plate R*.

Test solution Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 2 mL with the same mixture of solvents.

Reference solution (a) Dissolve 20 mg of *digitoxin CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 2 mL with the same mixture of solvents.

Reference solution (b) Dilute 0.5 mL of reference solution (a) to 50 mL with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (c) Dissolve 10 mg of *gitoxin CRS* with stirring in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 50 mL with the same mixture of solvents.

Reference solution (d) Dilute 1 mL of reference solution (b) to 2 mL with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Reference solution (e) Mix 1 mL of reference solution (a) and 1 mL of reference solution (c).

Apply to the plate 5 µL of each solution. Develop immediately over a path of 15 cm using a mixture of 15 volumes of *methanol R*, 40 volumes of *cyclohexane R* and 90 volumes of *methylene chloride R*. Dry the plate in a stream of cold air for 5 min. Repeat the development and dry the plate in a stream of cold air for 5 min. Spray with a mixture of 1 volume of *sulfuric acid R* and 9 volumes of *alcohol R* and heat at 130 °C for 15 min. Examine in daylight.

Gitoxin Any spot corresponding to gitoxin in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c) (2.0 per cent).

Other glycosides Any spot in the chromatogram obtained with the test solution, apart from the principal spot and the spot corresponding to gitoxin, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

The test is not valid unless the chromatogram obtained with reference solution (e) shows clearly separated spots corresponding to digitoxin, gitoxin and other glycosides and the spot in the chromatogram obtained with reference solution (d) is clearly visible.

Loss on drying (2.2.32)

Not more than 1.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on the residue from the test for loss on drying.

ASSAY

Dissolve 40.0 mg in *alcohol R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *alcohol R*. Prepare a reference solution in the same manner, using 40.0 mg of *digitoxin CRS*. To 5.0 mL of each solution add 3.0 mL of *alkaline sodium picrate solution R*, allow to stand protected from bright light for 30 min and measure the absorbance (*2.2.25*) of each solution at the maximum at 495 nm, using as the compensation liquid a mixture of 5.0 mL of *alcohol R* and 3.0 mL of *alkaline sodium picrate solution R* prepared at the same time.

Calculate the content of $C_{41}H_{64}O_{13}$ from the absorbances measured and the concentrations of the solutions.

STORAGE

Store protected from light.

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