Dissolution ⟨711⟩—[NOTE—Throughout this procedure, use scrupulously clean glassware, which previously has been rinsed successively with hydrochloric acid, water, and alcohol, and carefully dried. Take precautions to prevent contamination from fluorescent particles and from metal and rubber sur faces.]

Medium: dilute hydrochloric acid (3 in 500); 500 mL. [NOTE—Use the same batch of *Medium* throughout the test.]

Apparatus 1: 120 ± 5 rpm.

Times: 30 minutes; 60 minutes.

Standard stock solution—Weigh accurately about 30 mg of USP Digitoxin RS, dissolve in a minimum amount of alcohol in a 500-mL volumetric flask, add dilute alcohol (4 in 5) to volume, and mix.

Standard solutions—Just prior to use, dilute 5.0 mL of the *Standard stock solution* with *Medium* to 500.0 mL, and mix. Transfer aliquots (2.0 to 10.0 mL) of this solution to individual separators to prepare standards equivalent to 20, 40, 60, 80, and 100% of the labeled amount of digitoxin in 500 mL. Add *Medium* to make 10 mL, and proceed as directed for *Procedure*, beginning with "Extract with three 15-mL portions of chloroform."

Procedure—Proceed as directed for *Procedure* under *Dissolution* ⟨711⟩. After 30 minutes, accurately timed, withdraw a suitable aliquot of the solution under test from a point midway between the stirring shaft and the wall of the vessel, and approximately midway in depth. Filter the solution promptly after withdrawal, using a suitable membrane filter of not greater than 0.8-µm porosity, discarding the first 10 mL of the filtrate. Without replacing the *Medium* withdrawn, continue to rotate the basket, and after an additional 30 minutes, accurately timed, similarly withdraw and filter another aliquot. T reat each of these solutions as follows: Assuming dissolution of 100% of the labeled amount of digitoxin, transfer aliquots, equivalent to 6 µg of digitoxin, to suitable separators. Extract with three 15-mL portions of chloroform, and combine the chloroform extracts in glass-stoppered flasks. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dr yness. In a similar manner, prepare a blank using a suitable volume of *Medium*.

Measurement of fluorescence—Begin with the *Standard solutions,* and keep all flasks in the same sequence throughout, so that the elapsed time from addition of reagents to reading of fluorescence is the same for each set. T reat 1 flask at a time as follows: Add 10 mL of a solution freshly prepared by dissolving 35 mg of ascorbic acid in 25 mL of methanol and cautiously adding the solution to 100 mL of hydrochloric acid. Mix, and add 1 mL of a solution freshly prepared by diluting 1 mL of 30 percent hydrogen peroxide with water to 500 mL and diluting 1 volume of the resulting solution with 20 volumes of water. Mix, and insert the stopper in the flask. After 45 minutes, measure the fluorescence at about 575 nm, the excitation wavelength being about 395 nm. Correct each reading for the blank, and plot a standard cur ve of fluorescence versus per centage dissolution. By calculation from the standard cur ve, determine the per centage dissolution of digitoxin in each T ablet within 30 minutes and the total per centage dissolution of digitoxin within 60 minutes, taking into account the volume of the solution under test removed after the first 30 minutes of the test.

Tolerances—Not less than 60% of the labeled amount of $C_{41}H_{64}O_{13}$ is dissolved within 30 minutes for each T ablet tested, and not less than 85% of the labeled amount is dissolved within 60 minutes for the average of the T ablets tested.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Place 1 Tablet in a suitable glass-stoppered conical flask. Add an accurately measured volume of *Mobile phase* (prepared as directed in the *Assay* under *Digitoxin*) sufficient to obtain a solution containing about 10 µg of digitoxin per mL, and shake by mechanical means until the Tablet has completely disintegrated (not less than 30 minutes). Centrifuge, and use the clear supernatant as the test solution. Dissolve an accurately weighed quantity of USP Digitoxin RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a Standard solution having a known concentration of about 10 µg per mL. Proceed as directed in the *Assay*. Calculate the quantity, in mg, of $C_{41}H_{64}O_{13}$ in the Tablet by the formula:

$$(LC / D)(r_U / r_S)$$

in which L is the labeled quantity, in mg, of digitoxin in the Tablet, C is the concentration, in µg per mL, of USP Digitoxin RS in the Standard solution, D is the concentration, in µg per mL, of digitoxin in the test solution based on the labeled quantity in the Tablet and the extent of dilution, and r_U and r_S are the digitoxin peak responses obtained from the test solution and the Standard solution, respectively.

Assay—

Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Digitoxin*.

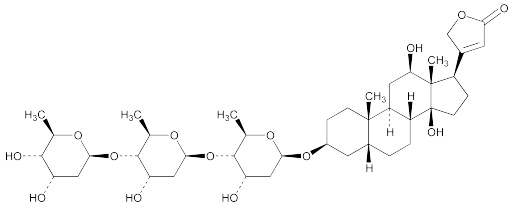
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of powder, equivalent to about 1 mg of digitoxin, to a 25-mL volumetric flask. Add 15 mL of *Mobile phase*, and sonicate. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution, discarding the first few mL of the filtrate. The filtrate is the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Digitoxin*. Calculate the quantity, in µg, of $C_{41}H_{64}O_{13}$ in the portion of Tablets taken by the formula:

$$25C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Digitoxin RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Digoxin



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

Card-20(22)-enolide, 3-[(*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]-12,14-dihydroxy-, 3*β*,5*β*,12*β*)-.

Digoxin.

3*β*-[(*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]-12*β*,14-dihydroxy-5*β*-card-20(22)-enolide [20830-75-5].

» Digoxin is a cardiotonic glycoside obtained from the leaves of *Digitalis lanata* Ehrhart (Fam. Scrophulariaceae). It contains not less than 95.0 percent and not more than 101.0 per cent of $C_{41}H_{64}O_{14}$, calculated on the dried basis.

Caution—Handle Digoxin with exceptional care, since it is extremely poisonous.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Digoxin RS
USP Gitoxin RS
$C_{41}H_{64}O_{14}$ 780.96

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

C: Examine in visible light the thin-layer chromatograph prepared as directed in the test for *Related glycosides:* the R_F value of the principal blue spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

Loss on drying ⟨731⟩**—**Dry it in vacuum at 105 ° for 1 hour: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%, a 100-mg specimen being used.

Related glycosides—

Chloramine T–trichloroacetic acid reagent—Mix 10 mL of a freshly prepared solution of chloramine T (3 in 100) and 40 mL of a 1 in 4 solution of trichloroacetic acid in dehydrated alcohol.

Spotting solvent—Prepare a mixture of chloroform and methanol (2:1).

Standard solution—Dissolve an accurately weighed quantity of USP Digoxin RS in *Spotting solvent* to obtain a solution containing 10 mg per mL.

Gitoxin standard solution—Dissolve an accurately weighed quantity of USP Gitoxin RS in *Spotting solvent* to obtain a solution containing 0.30 mg per mL.

Test solution—Transfer 250.0 mg of Digoxin to a 25-mL volumetric flask, dissolve in and dilute with *Spotting solvent* to volume, and mix.

Procedure—Apply 10 µL of the *Test solution*, 10 µL of the *Standard solution*, and 10 µL of the *Gitoxin standard solution* on a line parallel to and about 2.5 cm from the bottom edge of a reversed-phase thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture to which is permanently bonded octadecylsilane (C18). Allow the spots to dry, and place the plates in a developing chamber containing a mixture of methanol and water (7:3). Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Chloramine T–trichloroacetic acid reagent*, freshly mixed, and heat in an oven at 110 ° for 10 minutes. Examine the plate under long-wavelength UV light: no spot from the *Test solution* except that due to digoxin is more intense than the spot from the *Gitoxin standard solution* (not more than 3% of any related glycoside as gitoxin).

Residual solvents ⟨467⟩**:** The limits for methylene chloride and chloroform are 2000 µg per g.

Assay—

Mobile phase—Prepare a suitable degassed and filtered mixture of water and acetonitrile (37:13), making adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively and stepwise with diluted alcohol to obtain a solution having a known concentration of about 250 µg per mL. Use a sonic bath to aid dissolution.

Assay preparation—Transfer about 50 mg of Digoxin, accurately weighed, to a 200-mL volumetric flask. Dissolve in about 150 mL of diluted alcohol by sonication, dilute with diluted alcohol to volume, and mix.

System suitability preparation—Prepare a solution in diluted alcohol of USP Digoxin RS and digoxigenin having concentrations of about 40 µg of each per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 218-nm detector and a 4.2-mm × 25-cm column that contains packing L1 and a 3.2-mm × 15-mm guard column that contains packing L1. The flow rate is about 3.0 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between digoxin and digoxigenin is not less than 4.0; the column efficiency determined from the digoxin peak is not less than 1200 theoretical plates; the tailing factor for the digoxin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{41}H_{64}O_{14}$ in the portion of Digoxin taken by the formula:

$$0.2C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Digoxin RS in the *Standard preparation;* and r_U and r_S are the responses for the digoxin peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Digoxin Injection

» Digoxin Injection is a sterile solution of Digoxin in Water for Injection and Alcohol or other suitable solvents. It contains not less than 90.0 percent and not more than 105.0 per cent of the labeled amount of $C_{41}H_{64}O_{14}$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass. Avoid exposure to excessive heat.

USP Reference standards ⟨11⟩**—**
USP Digoxin RS
USP Endotoxin RS

Identification—

A: Injection meets the requirements for *Identification* test *A* under *Digoxin Oral Solution.*

B: *Chloramine T–trichloroacetic acid reagent, Spotting solvent,* and *Standard solution*—Proceed as directed for *Identification* test *B* under *Digoxin Oral Solution*.

Test solution—Pipet a volume of Injection, equivalent to 0.5 mg of digoxin, into a separator, and add 5 mL of water. Extract with three 10-mL portions of chloroform, combining the extracts in a conical flask. Evaporate the combined chloroform extracts on a steam bath with the aid of a current of air to dryness. (If traces of water or propylene glycol remain, dr y the flask in vacuum at 100 ° for 30 minutes.) Dissolve the residue in 2 mL of *Spotting solvent*.

Procedure—Proceed as directed for *Procedure* in the test for *Related glycosides* under *Digoxin,* except to omit the use of the *Gitoxin standard solution*. Examine the plate under long-wavelength UV light: the R_F value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard solution*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 200.0 USP Endotoxin Units per mg of digoxin.

Alcohol content ⟨611⟩: between 9.0% and 11.0% of C_2H_5OH.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Mobile phase—Proceed as directed in the *Assay* under *Digoxin*.