

methanol by warming, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Dexamethasone as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution obtained responds to the Qualitative Tests for fluoride.

(4) Dissolve 1.0 mg of Dexamethasone in 10 mL of ethanol (95). Mix 2.0 mL of the solution with 10 mL of phenylhydrazine hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, using as the blank the solution prepared with 2.0 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dexamethasone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared absorption spectrum of Dexamethasone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Dexamethasone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Dexamethasone and Dexamethasone Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation $[\alpha]_D^{20}$: +72 – +80° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Heavy metals—Proceed with 1.0 g of Dexamethasone according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Other steroids—Dissolve 0.10 g of Dexamethasone in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μ L 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 dichloromethane and methanol (45:4) 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% (0.2 g, 105°C, 3 hours).

Residue on ignition Not more than 0.1% (0.2 g, platinum crucible).

Assay Dissolve about 0.01 g each of Dexamethasone and Dexamethasone Reference Standard, previously dried and accurately weighed, in 70 mL each of diluted methanol (1 in 2), add exactly 5 mL each of the internal standard solution, then add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of these solutions 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 dexamethasone to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of } C_{22}H_{29}FO_5 \\ &= \text{amount (mg) of Dexamethasone} \\ & \quad \text{Reference Standard} \\ & \quad \times \frac{Q_T}{Q_S} \end{aligned}$$

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (2:1).

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 6 minutes.

Selection of column: Dissolve 2 mg of methyl parahydroxybenzoate and 4 mg of ethyl parahydroxybenzoate in 100 mL of diluted methanol (1 in 2). Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of methyl parahydroxybenzoate and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dextran 40

デキストラン 40

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 40,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 40.

Description Dextran 40 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 40 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH Dissolve 1.0 g of Dextran 40 in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

(2) Chloride—Perform the test with 2.0 g of Dextran 40. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid (not more than 0.018%).

(3) Heavy metals—Proceed with 1.0 g of Dextran 40 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic—Prepare the test solution with 1.5 g of Dextran 40 according to Method 1, and perform the test using Apparatus B (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 40, previously dried, and perform the test as directed under the Nitrogen Determination, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.01) is not more than 0.010%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.450 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL each of these solutions, add 5 mL of alkaline copper TS, exactly measured, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying Not more than 5.0% (1 g, 105°C, 6 hours).

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

Viscosity (1) Dextran 40—Weigh accurately 0.2 to 0.5 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 under the Viscosity Determination at 25°C: the intrinsic viscosity is between 0.16 and 0.19.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually 80 to 90 mL) at 25 ± 1°C with stirring. Dissolve the precipitate at 35°C in a water bath with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not more than 0.27.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 115 to 135 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determined the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.09.

Antigenicity Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Pyrogen Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, and perform the test: this solution meets the requirements of the Pyrogen Test.

Assay Weigh accurately about 3 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D with the sample solution as directed under the Optical Rotation Determination in a 100-mL cell at 20 ± 1°C.

$$\text{Amount (mg) of dextran 40} = \alpha_D \times 253.8$$

Containers and storage Containers—Tight containers.

Dextran 40 Injection

デキストラン 40 注射液

Dextran 40 Injection is an aqueous solution for injection. It contains not less than 9.5 w/v% and not more than 10.5 w/v% of dextran 40.

Method of preparation

Dextran 40	10 g
Isotonic Sodium Chloride Solution	a sufficient quantity
To make 100 mL	

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Dextran 40 Injection is a clear and colorless liquid. It is slightly viscous.

Identification (1) Dilute 1 mL of Dextran 40 Injection with water to 200 mL, and to 1 mL of the diluted solution add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution does not change in color.

(2) Dextran 40 Injection responds to the Qualitative