Assay—

Mobile phase, pH 6.0 Buffer solution, Diluent, and *Chromatographic system*—Proceed as directed in the *Assay* under *Dexamethasone Acetate*.

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone Acetate RS in *Diluent* to obtain a solution having a known concentration of about 0.09 mg per mL.

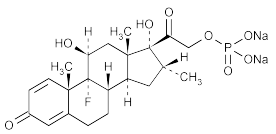
Assay preparation—Transfer an accurately measured volume of well-shaken Injectable Suspension, equivalent to about 40 mg of dexamethasone, to a 100-mL volumetric flask. Add 75 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* (before and after injections of the *Assay 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 dexamethasone ($C_{22}H_{29}FO_5$) in each mL of the Injectable Suspension taken by the formula:

$$(392.47 / 434.51)(500C / V)(r_U / r_S)$$

in which 392.47 and 434.51 are the molecular weights of dexamethasone and anhydrous dexamethasone acetate, respectively; C is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation; V* is the volume, in mL, of Injectable Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dexamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$ 516.41

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy)-, disodium salt, (11 β,16α)-.

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-(dihydrogen phosphate) disodium salt [2392-39-4].

» Dexamethasone Sodium Phosphate contains not less than 97.0 per cent and not more than 102.0 percent of $C_{22}H_{28}FNa_2O_8P$, calculated on the water-free and alcohol-free basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**

USP Dexamethasone RS

USP Dexamethasone Phosphate RS

Identification—

A: *pH 9 Buffer with magnesium*—Mix 3.1 g of boric acid and 500 mL of water in a 1-L volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix.

Alkaline phosphatase solution—Transfer 95 ± 5 mg of alkaline phosphatase enzyme to a 50-mL volumetric flask, dissolve by adding *pH 9 Buffer with magnesium* to volume, and mix. Prepare this solution fresh daily.

Standard solution—Weigh 15 mg of USP Dexamethasone RS into a 5-mL volumetric flask. Dissolve in and dilute with ethyl acetate to volume. [NOTE—Sonication may be required to ensure dissolution.]

Test solution—Weigh 20 mg of Dexamethasone Sodium Phosphate into a 15-mL centrifuge tube. Add 5.0 mL of *Alkaline phosphatase solution,* shake vigorously, and allow to stand for 30 minutes. Add 5.0 mL of ethyl acetate, shake vigorously, centrifuge, and use the upper, ethyl acetate layer.

Procedure—Apply 10-µL portions of the *Test solution* and the *Standard solution* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a mobile phase consisting of a mixture of chloroform, methanol, and water (180:15:1) to a distance of three-fourths of the length of the plate. Air-dry the plate and observe under short-wavelength UV light: the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

B: The residue from the ignition of it meets the requirements of the tests for *Phosphate* ⟨191⟩ and for *Sodium* ⟨191⟩.

Specific rotation ⟨781S⟩: between +74° and +82°, calculated on the water-free and alcohol-free basis.

Test solution: 10 mg per mL, in water.

pH ⟨791⟩: between 7.5 and 10.5, in a solution (1 in 100).

Water, *Method I* ⟨921⟩**—**Determine the water content. The sum of the percentages of water content, and alcohol content, determined as directed in the test for *Alcohol*, does not exceed 16.0%.

Limit of phosphate ions—

Standard phosphate solution—Dissolve 143.3 mg of dried monobasic potassium phosphate, KH_2PO_4, in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate (PO_4) in each mL.

Phosphate reagent A—Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.

Phosphate reagent B—Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium bisulfite, mix to dissolve, and dilute with water to 100 mL.

Procedure—Dissolve about 50 mg of Dexamethasone Sodium Phosphate, accurately weighed, in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25 mL, mix, and allow to stand at room temperature for 30 minutes. Similarly and concomitantly, prepare a standard solution, using 5.0 mL of *Standard phosphate solution* instead of the 50 mg of the substance under test. Concomitantly determine the absorbances of both solutions in 1-cm cells at 730 nm, with a suitable spectrophotometer, using water as the blank. The absorbance of the test solution is not more than that of the standard solution. The limit is 1.0% of phosphate (PO_4).

Limit of free dexamethasone—

Mobile phase—Prepare a solution containing 7.5 mL of triethylamine in 1 L of water. Adjust by the addition of phosphoric acid to a pH of 5.4. Prepare a filtered and degassed mixture of 74 parts of the resulting solution with 26 parts of methanol. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Dexamethasone Phosphate RS in *Mobile phase* to obtain a solution containing about 0.5 mg per mL. Prepare a second solution by dissolving an accurately weighed quantity of USP Dexamethasone RS in a mixture of methanol and water (1:1) to obtain a solution containing about 50 µg per mL. Transfer 10.0 mL of the first solution and 1.0 mL of the second solution to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution having known concentrations of 50 µg of USP Dexamethasone Phosphate RS per mL and 0.5 µg of USP Dexamethasone RS per mL.

Test solution—Transfer about 50 mg of Dexamethasone Sodium Phosphate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and

mix. Further dilute 5.0 mL of this solution with *Mobile phase* to 50.0 mL.

System suitability solution—Prepare a solution in *Mobile phase* containing in each mL 0.05 mg of USP Dexamethasone Phosphate RS and 0.02 mg of USP Dexamethasone RS .

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.5-mm × 25-cm column that contains 5- µm packing L11. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard solution* and the *System suitability solution*, record the peak responses as directed for *Procedure*, and determine the chromatographic characteristics from chromatograms obtained from the *System Suitability:* the column efficiency determined from the analyte peak is not less than 900 theoretical plates; the tailing factor for the analyte peak is not more than 1.6; the resolution, *R,* between dexamethasone phosphate and dexamethasone is not less than 1.8; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the dexamethasone peaks. Calculate the quantity, in µg, of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Dexamethasone Sodium Phosphate taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Dexamethasone RS in the *Standard solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is found.

Chromatographic purity—

Acetate buffer—Dissolve 7 g of ammonium acetate in 1 L of water, adjust with glacial acetic acid to a pH of 4.0, and mix.

Solution A—Prepare a filtered and degassed mixture of methanol, water, and *Acetate buffer* (7:7:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solution B—Prepare a filtered and degassed mixture of methanol and *Acetate buffer* (7:3). Make adjustments if necessar y (see *System Suitability* under *Chromatography* ⟨621⟩).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.*

Test solution—Transfer about 25 mg of Dexamethasone Sodium Phosphate, accurately weighed, to a 25-mL volumetic flask, dissolve in and dilute with *Solution A* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	90	10	equilibration
0–3.5	90	10	isocratic
3.5–23.5	90→60	10→40	linear gradient
23.5–34.5	60→5	40→95	linear gradient
34.5–59.5	5	95	isocratic
59.5–60	5→90	95→10	linear gradient

Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the resolution between the major peak and the nearest impurity is not less than 1.0; and the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 15 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the per centage of each impurity in the portion of Dexamethasone Sodium Phosphate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all peaks: not more than 1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

Alcohol content, *Method II* ⟨611⟩**—**Proceed as directed in the chapter except to use column packing S8 and to use the following modifications.

Internal standard solution—Pipet 1 mL of isopropyl alcohol into a 100-mL volumetric flask, add water to volume, and mix.

Standard stock solution—Prepare a solution of alcohol in water (1 in 50). Determine the specific gravity at 25 ° (see *Specific* ⟨841⟩), and obtain the per centage of C_2H_5OH by reference to the *Alcoholometric Table* in the section *Reference Tables*.

Standard solution—Into a 10-mL volumetric flask pipet 4 mL of *Standard stock solution* and 5 mL of *Internal standard solution*, add water to volume, and mix. Inject 2 µL of this solution into the gas chromatograph.

Test solution—Transfer about 500 mg of Dexamethasone Sodium Phosphate, accurately weighed, into a 10-mL volumetric flask. Pipet 5 mL of *Internal standard solution* into the flask, and mix to dissolve. Add water to volume, and mix. Inject 2 µL of this solution into the gas chromatograph.

Calculation—Calculate the per centage of alcohol in the Dexamethasone Sodium Phosphate taken by the formula:

$$4(S / W)(Z / Y)$$

in which S is the per centage of alcohol in the *Standard stock solution; W* is the weight, in g, of Dexamethasone Sodium Phosphate used in the *Test solution;* and Y and Z are the ratios of the alcohol peak heights to the internal standard peak heights for the *Standard solution* and the *Test solution*, respectively. The content of C_2H_5OH is not more than 8.0%.

Assay—

Buffer solution—Dissolve 7.0 g of ammonium acetate in 1 L of water, adjust with glacial acetic acid to a pH of 4.00 ± 0.05, and mix.

Solution A—Prepare a filtered and degassed mixture of methanol, water, and *Buffer solution* (350:350:300).

Solution B—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (700:300).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessar y (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone Phosphate RS in *Solution A* to obtain a solution having a known concentration of about 0.92 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Dexamethasone Sodium Phosphate in *Solution A,* and mix to obtain a solution having a concentration of about 1.0 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The column temperature is maintained at about 40 °. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	90	10	equilibration
0–3.5	90	10	isocratic
3.5–24	90→60	10→40	linear gradient
24–35	60→5	40→95	linear gradient

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
35–60	5	95	isocratic
60–60.1	5→90	95→10	linear gradient
60.1–65	90	10	isocratic

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%. Chromatograph the *Assay preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between dexamethasone phosphate and the nearest impurity eluting after it is not less than 1.0.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{28}FNa_2O_8P$ in the portion of Dexamethasone Sodium Phosphate taken by the formula:

$$(516.41 / 472.45)C(r_U / r_S)$$

in which 516.41 and 472.45 are the molecular weights of dexamethasone sodium phosphate and dexamethasone phosphate, respectively; *C* is the concentration, in mg per mL, of USP Dexamethasone Phosphate 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.

Dexamethasone Sodium Phosphate Inhalation Aerosol

» Dexamethasone Sodium Phosphate Inhalation Aerosol is a suspension, in suitable propellants and alcohol, in a pressurized container, of dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).

Packaging and storage—Preserve in tight, pressurized containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩**—**
USP Dexamethasone RS

Identification—Prepare a pH 9.0 buffer solution by dissolving 3.1 g of boric acid, 203 mg of magnesium chloride, and 860 mg of sodium hydroxide in water to make 1000 mL. Dissolve 50 mg of alkaline phosphatase enzyme in 50 mL of the pH 9.0 buffer solution, and transfer 5 mL of the resulting solution to a glass-stoppered, 50-mL tube containing 5 mL of the *Assay preparation* prepared as directed in the *Assay*. Incubate at 37 ° for 45 minutes, add 25 mL of methylene chloride, and shake for 2 minutes: the methylene chloride extract so obtained responds to the *Identification* test under *Dexamethasone Sodium Phosphate Injection*, beginning with "Evaporate 15 mL of the methylene chloride extract."

Alcohol content, *Method II* ⟨611⟩: between 1.7% and 2.3% of C_2H_5OH.

Delivered dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Standard solution—Transfer about 10 mg of USP Dexamethasone RS, accurately weighed, to a 10-mL volumetric flask, dilute with alcohol to volume, and mix. T ransfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix to obtain a solution having a known concentration of about 10 µg per mL.

Test solution—Discharge the minimum recommended dose into the sampling apparatus, and detach the inhaler as directed. Rinse the apparatus (filter and interior) with two 5.0-mL portions of 0.1 N sulfuric acid, and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube containing 15 mL of methylene chloride that was previously chilled in a dr y ice-acetone bath for a few minutes. Insert the stopper in the centrifuge tube, and shake cautiously, releasing the pressure occasionally. Allow the phases to separate, and equilibrate to room temperature. The aqueous phase is the *Test solution*.

Procedure—Transfer the *Test solution* and 10.0 mL of the *Standard solution* into separate flasks. Add 2.0 mL of 0.1 N sulfuric acid to each, and swirl to mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in µg, of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) contained in the minimum dose by the formula:

$$10(472.45 / 392.47)(CN)(A_U / A_S)$$

in which 472.45 and 392.47 are the molecular weights of dexamethasone phosphate and dexamethasone, respectively; *C* is the concentration, in µg per mL, of USP Dexamethasone RS in the *Standard solution; N* is the number of sprays discharged to obtain the minimum recommended dose; and A_U and A_S are the absorbances of the solutions from the *Test solution* and the *Standard solution,* respectively.

Assay—

Standard preparation—Transfer about 40 mg of USP Dexamethasone RS, accurately weighed, to a 50-mL volumetric flask, dilute with alcohol to volume, and mix. T ransfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix to obtain a solution having a known concentration of about 8 µg per mL.

Assay preparation—Weigh accurately a filled Inhalation Aerosol container, and record the weight (W_1). Place the container in a dr y ice-acetone bath, and cool for 60 minutes. Remove the container from the bath, and carefully remove the valve with wire cutters, taking precautions to save all pieces of the valve and cap. With the aid of four 5-mL portions of 0.1 N sulfuric acid, transfer the contents of the container to a beaker previously chilled in the bath. Dr y the rinsed empty container and all of its parts in an oven at 105 ° for 2 hours, cool, and weigh (W_2). Allow the contents of the beaker to warm to room temperature. After the bulk of the propellant has evaporated, quantitatively transfer the contents of the beaker, with the aid of several mL of 0.1 N sulfuric acid, to a 200-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix. T ransfer about 20 mL of this solution to a centrifuge tube, add 10 mL of methylene chloride, shake vigorously for 1 minute, and centrifuge. Pipet 10 mL of the clear supernatant into a 100-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in each g of Inhalation Aerosol taken by the formula:

$$2(472.45 / 392.47)(A_U / A_S)[C / (W_1 - W_2)]$$

in which 472.45 and 392.47 are the molecular weights of dexamethasone phosphate and dexamethasone, respectively, A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively, *C* is the concentration, in µg per mL, of USP Dexamethasone RS in the *Standard preparation*, and W_1 and W_2 are the weights, in g, as previously defined.