solutions in 1-cm cells at the wavelength of maximum absorbance at about 408 nm, with a suitable spectrophotometer, using the *Blank* to set the instrument.

Calculate the percentage of the label claim of $C_{21}H_{29}NO \cdot HCl$ in the Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Biperiden Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 45 min

[NOTE—Determine the amount of $C_{21}H_{29}NO \cdot HCl$ dissolved by using the following method.]

Phosphate buffer–bromocresol purple solution: Prepare as directed in the *Assay*.
Standard stock solution: 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol
Standard solution: 2 µg/mL of USP Biperiden Hydrochloride RS, prepared as follows: Pipet 5 mL of *Standard stock solution* into a 500-mL volumetric flask, and add 0.01 N hydrochloric acid to volume. Pipet 25 mL of this solution into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.
Sample solution: Sample per *Dissolution* ⟨711⟩. Filter 75 mL of the solution under test, pipet 50 mL of the clear filtrate into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.
Blank: Water
Analysis
Samples: *Standard solution, Sample solution*, and *Blank*
Pipet 20.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* into individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 40.0 mL of chloroform for 10 min. After the layers have separated, pass each chloroform extract through filter paper into separate, glass-stoppered containers, discarding the first 10 mL of each filtrate. Determine the amount of $C_{21}H_{29}NO \cdot HCl$ dissolved from absorbances at the wavelength of maximum absorbance at about 408 nm (10-cm cells) of the extract from the *Sample solution* in comparison with that of the extract from the *Standard solution*, using the *Blank* to set the instrument.
Tolerances: NLT 75% (Q) of $C_{21}H_{29}NO \cdot HCl$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Biperiden Hydrochloride RS

Biperiden Lactate Injection

$C_{21}H_{29}NO \cdot C_3H_6O_3$ 401.54
1-Piperidinepropanol, α-bicyclo[2.2.1]hept-5-en-2-yl-α-phenyl-, compd. with 2-hydroxypropanoic acid (1:1).
α-5-Norbornen-2-yl-α-phenyl-1-piperidinepropanol lactate (salt) [7085-45-2].

» Biperiden Lactate Injection is a sterile solution of biperiden lactate ($C_{21}H_{29}NO \cdot C_3H_6O_3$) in Water for Injection, prepared from Biperiden with the aid of Lactic Acid. It contains not less than 95.0 percent and not more than 105.0 per cent of the labeled amount of $C_{21}H_{29}NO \cdot C_3H_6O_3$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Biperiden RS
USP Endotoxin RS

Identification—Using a volume of Injection, equivalent to about 50 mg of biperiden lactate, and using a solution of 50 mg of USP Biperiden RS in 25 mL of 0.01 N hydrochloric acid, proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator": the Injection meets the requirements of the test.

Bacterial endotoxins ⟨85⟩—It contains not more than 83.3 USP Endotoxin Units per mg of biperiden lactate.

pH ⟨791⟩: between 4.8 and 5.8.

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

Assay—

Phosphate buffer–bromocresol purple solution—Prepare as directed in the *Assay* under *Biperiden Hydrochloride Tablets.*

Standard preparation—Transfer about 80 mg of USP Biperiden RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, add 25 mL of water, dilute with methanol to volume, and mix to obtain a *Standard preparation* having a known concentration of about 40 µg per mL.

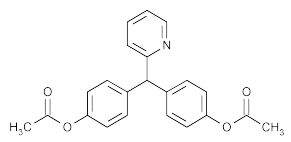
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of biperiden lactate, to a 100-mL volumetric flask, add 25 mL of water, dilute with methanol to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Biperiden Hydrochloride Tablets.* Calculate the quantity, in mg, of $C_{21}H_{29}NO \cdot C_3H_6O_3$ in each mL of the Injection taken by the formula:

$$(401.55 / 311.47)(0.1C / V)(A_U / A_S)$$

in which 401.55 and 311.47 are the molecular weights of biperiden lactate and biperiden, respectively; C is the concentration, in µg per mL, of USP Biperiden RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Bisacodyl



$C_{22}H_{19}NO_4$ 361.39
Phenol, 4,4′-(2-pyridinylmethylene)bis-, diacetate (ester).
4,4′-(2-Pyridylmethylene)diphenol diacetate (ester) [603-50-9].

» Bisacodyl contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{22}H_{19}NO_4$, calculated on the dried basis.

Caution—Avoid inhalation and contact with the eyes, skin, and mucous membranes.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Bisacodyl RS

Identification—

A: *Infrared Absorption* ⟨197S⟩—

Cell: 1.0 mm.

Solution: 1 in 200 solution in chloroform, previously dried.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 20 µg per mL.

Medium: 0.05 N hydrochloric acid.

Absorptivities at 263 nm, calculated on the dried basis, do not differ by more than 3.0%.

Melting range ⟨741⟩: between 131° and 135°.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: 0.001%.

Assay—Dissolve about 250 mg of Bisacodyl, accurately weighed, in 70 mL of glacial acetic acid, add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.14 mg of $C_{22}H_{19}NO_4$.

Bisacodyl Suppositories

» Bisacodyl Suppositories contain not less than 90.0 percent and not more than 110.0 per cent of the labeled amount of $C_{22}H_{19}NO_4$.

Packaging and storage—Preserve in well-closed containers at a temperature not exceeding 30°.

USP Reference standards ⟨11⟩—
USP Bisacodyl RS

Identification—

A: Transfer a quantity of Suppositories, equivalent to about 150 mg of bisacodyl, to a 500-mL conical flask, add 75 mL of solvent hexane, and heat on a steam bath until they are melted. Filter the solution, with the aid of vacuum, through a medium-porosity, sintered-glass funnel, and wash the residue with about 100 mL of warm solvent hexane until it is free from fat. Continue the vacuum until the residue appears dry. Dissolve the residue by rinsing the filter with about 50 mL of warm acetone, collecting the filtrate in a 150-mL beaker, and evaporate the filtrate on a steam bath to a volume of about 5 mL. To the residual liquid add about 75 mL of water, heat on a steam bath for 15 minutes, and cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 minutes: the bisacodyl so obtained melts between 129° and 135°, and responds to *Identification* test *A* under *Bisacodyl.*

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for bisacodyl, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation.*

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.074 M sodium acetate in water [adjusted with 2.5% (v/v) acetic acid to a pH of 7.4] and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Bisacodyl RS in acetonitrile to obtain a *Standard preparation* having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer a number of Suppositories, equivalent to about 100 mg of bisacodyl, to a 500-mL separator, add 150 mL of *n*-hexane, and shake until all the suppositories are dissolved. Add 50 mL of acetonitrile, shake for 1 minute, and allow the layers to separate. Drain the lower layer into a 200-mL volumetric flask, and extract the *n*-hexane layer remaining in the separator with two 50-mL portions of acetonitrile, combining the lower layers in the volumetric flask. Dilute the combined extracts in the volumetric flask with acetonitrile to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 265-nm detector, a 3.9-mm × 30-cm column that contains packing L1, and a guard column that contains packing L2. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor 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 $C_{22}H_{19}NO_4$ in the Suppositories taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Bisacodyl 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.

Bisacodyl Rectal Suspension

» Bisacodyl Rectal Suspension is a suspension of Bisacodyl in a suitable aqueous medium. It contains not less than 90.0 per cent and not more than 115.0 percent of the labeled amount of $C_{22}H_{19}NO_4$.

Packaging and storage—Preserve in unit-dose containers at a temperature not exceeding 30°.

USP Reference standards ⟨11⟩—
USP Bisacodyl RS

Identification—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.*

pH ⟨791⟩: between 5.0 and 6.8.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol and 0.01 M monobasic potassium phosphate (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of ethylparaben in methanol, and dilute with an equal volume of water to obtain a solution containing about 5.0 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Bisacodyl RS in methanol, add an accurately measured volume of *Internal standard solution*, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 67 µg per mL and 250 µg per mL for bisacodyl and ethylparaben, respectively.

Assay preparation—Transfer an accurately measured volume of Rectal Suspension, equivalent to 6.7 mg of bisacodyl, to a