Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{27}H_{36}N_2O_4$ in the portion of Repaglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
  Ignition temperature: 600 ± 25°
- **HEAVY METALS,** *Method II* **⟨231⟩:** 10 ppm

Organic Impurities

- **PROCEDURE**
  Solution A: 3 mg/mL of monobasic potassium phosphate solution, adjusted with 1 N sodium hydroxide to a pH of 7.0
  Solution B: Methanol
  System suitability solution: A solution in methanol, containing 10 mg/mL of USP Repaglinide RS, 100 µg/mL of USP Repaglinide Related Compound A RS, 100 µg/mL of USP Repaglinide Related Compound B RS, and 100 µg/mL of USP Repaglinide Related Compound C RS
  Sample solution: 10 mg/mL of Repaglinide in methanol
  Standard solution: 0.1 mg/mL of repaglinide in methanol, from the *Sample solution*
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	50	50
2	30	70
8	30	70
12	5	95
15	5	95

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Column temperature: 45°
Injection size: 3 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for repaglinide related compound B, repaglinide related compound C, repaglinide, and repaglinide related compound A are 0.3, 0.9, 1.0, and 1.6, respectively.]
Suitability requirements
Relative standard deviation: NMT 10% of repaglinide, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Repaglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of repaglinide from the *Standard solution*
C_S = concentration of repaglinide in the *Standard solution* (mg/mL)
C_U = concentration of Repaglinide in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)
Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Repaglinide related compound A	1.6	2	0.1
Repaglinide related compound B	0.3	1	0.1
Repaglinide related compound C	0.9	1	0.1
Repaglinide	1.0	—	—
Total impurities	—	—	0.5

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* **⟨781S⟩:** +6.3° to +7.3°, at 20°
  Sample solution: 50 mg/mL, in methanol
- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Repaglinide RS
  USP Repaglinide Related Compound A RS
  (*S*)-3-Methyl-1-[2-(1-piperidinyl)phenyl]butylamine, *N*-acetyl-L-glutamate salt.
  $C_{16}H_{26}N_2 \cdot C_7H_{11}NO_5$ 435.6
  USP Repaglinide Related Compound B RS
  3-Ethoxy-4-ethoxycarbonylphenylacetic acid.
  $C_{13}H_{16}O_5$ 252.27
  USP Repaglinide Related Compound C RS
  (*S*)-2-Ethoxy-4-[2-[[2-phenyl-1-[2-(1-piperidinyl)phenyl]ethyl]amino]-2-oxoethyl]benzoic acid.
  $C_{30}H_{34}N_2O_4$ 486.61

Repaglinide Tablets

DEFINITION

Repaglinide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of repaglinide ($C_{27}H_{36}N_2O_4$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
  Sample solution: To a quantity of powdered Tablets, equivalent to 10 mg of repaglinide, add 10 mL of a mixture of methanol and methylene chloride (1:1), shake for 15 min, and centrifuge.
  Developing solvent system: Toluene, methylene chloride, and methanol (2:2:1)
- **B.** The retention time and UV spectrum of the major peak of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
  Solution A: Monobasic ammonium phosphate solution (2 in 1000). Adjust with phosphoric acid to a pH of 4.0.
  Solution B: Monobasic ammonium phosphate solution (2 in 1000). Adjust with phosphoric acid to a pH of 2.5.

Diluent: Methanol and *Solution A* (7:3)
Mobile phase: Methanol and *Solution B* (7:3)
Standard solution 1: 800 µg/mL of USP Repaglinide RS in methanol
Standard solution 2: Dilute 5.0 mL of *Standard solution 1* with *Diluent* to 50.0 mL.
System suitability stock solution: 80 µg/mL of USP Repaglinide Related Compound A RS in methanol
System suitability solution: Transfer 1.0 mL of *System suitability stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Standard solution 1*, and dilute with *Diluent* to volume.
Sample solution: Transfer 8 whole Tablets to a suitable volumetric flask, and dissolve in and dilute with *Diluent* to volume to obtain a solution containing 80 µg/mL. Stir for 20 min, and filter a portion of the solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 245-nm diode array
Column: 4.0-mm × 6-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution 2* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between repaglinide and repaglinide related compound A, *System suitability solution*
Capacity factors, k′: For repaglinide and repaglinide related compound A, about 4.9 and 1.2, respectively, *System suitability solution*
Tailing factor: 0.8–2.0, *System suitability solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution 2*
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of $C_{27}H_{36}N_2O_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution 2*
C_S = concentration of USP Repaglinide RS in *Standard solution 2* (mg/mL)
C_U = nominal concentration of repaglinide in the *Sample solution* (mg/mL)
Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨**711**⟩
Medium: pH 5.0 buffer, prepared by mixing 10.2 g of citric acid monohydrate and 18.16 g of dibasic sodium phosphate dihydrate with 1 L of water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Solution A: Monobasic potassium phosphate solution (1.5 in 1000), adjusted with phosphoric acid to a pH of 2.3
Mobile phase: Acetonitrile, *Solution A,* and methanol (49:40:11)
Standard stock solution: 11 µg/mL of USP Repaglinide RS in methanol
Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 25 mL of methanol, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Determine the amount of $C_{27}H_{36}N_2O_4$ dissolved by using the following method.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorometric detector; excitation wavelength of 244 nm and emission wavelength of 348 nm
Column: 4.0-mm × 12.5-cm; 10-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.5 and 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of $C_{27}H_{36}N_2O_4$ dissolved by comparing the measured peak responses from the *Standard solution* and the *Sample solution.*
Tolerances: NLT 70% (Q) of the labeled amount of $C_{27}H_{36}N_2O_4$ is dissolved.

• **Uniformity of Dosage Units** ⟨**905**⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **Procedure**
Solution A, Solution B, Diluent, Mobile phase, Standard solution 1, Standard solution 2, System suitability stock solution, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Standard solution 3: Dilute 2.5 mL of *Standard solution 2* with *Diluent* to 1000 mL.
Chromatographic system
Mode: LC
Detector: 210-nm diode array
Column: 4.0-mm × 6-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution 3* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between repaglinide and repaglinide related compound A, *System suitability solution*
Capacity factors, k′: For repaglinide and repaglinide related compound A, about 4.9 and 1.2, respectively, *System suitability solution*
Tailing factor: 0.8–2.0, *System suitability solution*
Relative standard deviation: NMT 10% for replicate injections, *Standard solution 3*
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

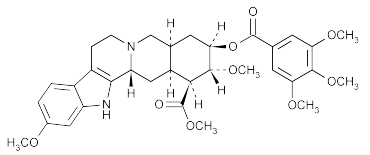
$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of repaglinide from the *Standard solution 2*
Acceptance criteria: NMT 0.5% of total impurities

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨**11**⟩
USP Repaglinide RS
USP Repaglinide Related Compound A RS
(*S*)-3-Methyl-1-[2-(1-piperidinyl)phenyl]butylamine,*N*-acetyl-L-glutamate salt.
$C_{16}H_{26}N_2 \cdot C_7H_{11}NO_5$ 435.6

Reserpine



$C_{33}H_{40}N_2O_9$ 608.68

Yohimban-16-carboxylic acid, 11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]-, methyl ester, (3*β*,16*β*,17*α*,18*β*,20*α*)-.

Methyl 18*β*-hydroxy-11,17*α*-dimethoxy-3*β*,20*α*-yohimban-16*β*-carboxylate 3,4,5-trimethoxybenzoate (ester) [50-55-5].

» Reserpine contains not less than 97.0 percent and not more than 101.0 percent of $C_{33}H_{40}N_2O_9$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

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

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: [NOTE—Conduct this test promptly, with a minimum exposure to light.] Dissolve 25.0 mg of it, previously dried, in 0.25 mL of chloroform; mix with about 30 mL of methanol previously warmed to 50°; transfer the mixture with the aid of warm methanol to a 250-mL volumetric flask; cool the solution to room temperature; dilute with methanol to volume; and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 36 mL of chloroform, dilute with methanol to volume, and mix: the UV absorption spectrum of a 1 in 50,000 solution so obtained exhibits the same maxima in the range of 255 nm to 350 nm as that of a similar solution of USP Reserpine RS, concomitantly measured; and the respective absorptivities, determined with reference to a mixture of 36 volumes of chloroform and 14 volumes of methanol as the blank, at the wavelength of maximum absorbance at about 268 nm, do not differ by more than 3.0%.

Loss on drying ⟨731⟩**—**Dry it at 60° for 3 hours: it loses not more than 0.5% of its weight.

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

Assay—

Mobile phase—Prepare a filtered and degassed 1:1 mixture of acetonitrile and ammonium chloride solution (1 in 100). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). The pH is about 5.6.

Standard preparation—Dissolve an accurately weighed quantity of USP Reserpine RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 10 µg per mL.

Assay preparation—Transfer about 10 mg of Reserpine, accurately weighed, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Dilute 1.0 mL of this solution with 9.0 mL of *Mobile phase,* and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 268-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 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 $C_{33}H_{40}N_2O_9$ in the portion of Reserpine taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Reserpine 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.

Reserpine Injection

» Reserpine Injection is a sterile solution of Reserpine in Water for Injection, prepared with the aid of a suitable acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{33}H_{40}N_2O_9$. It contains suitable antioxidants.

Packaging and storage—Preserve in single-dose (or, if stabilizers are present, in multiple-dose), light-resistant containers, preferably of Type I glass.

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

Identification—It responds to the *Identification* test under *Reserpine Oral Solution.*

Bacterial endotoxins ⟨85⟩**—**It contains not more than 71.5 USP Endotoxin Units per mg of reserpine.

pH ⟨791⟩: between 3.0 and 4.0.

Other alkaloids—[NOTE—Conduct this test promptly after preparation of the test and standard solutions.] Pipet 10 mL each of the citric acid solution of the Injection, and of *Solution 1* used in preparing the *Standard preparation,* respectively, obtained as directed in the *Assay*, into two separators. To the Injection solution add 100 mL of saturated sodium bicarbonate solution, and to *Solution 1* add 10 mL of water, 10 drops of saturated sodium bicarbonate solution, and 90 mL of water, and extract both of the resulting solutions with 50 mL of ether. Transfer the aqueous phase to another separator, extract with a second 50-mL portion of ether, and discard the aqueous layers. Wash the ether layers in succession with two 25-mL portions of water, and discard the washings. Extract the combined ether layers with three 15-mL portions of 2 N sulfuric acid, collect the extracts in a 50-mL volumetric flask, add 2 N sulfuric acid to volume, and mix. The absorption spectrum of the solution from the Injection, in the range of 255 to 350 nm, measured in a 1-cm cell, 2 N sulfuric acid being used as the blank, exhibits maxima and minima only at the same wavelengths as that of the solution from the *Standard preparation,* concomitantly measured. Calculate the quantity, in mg, of total alkaloids in each mL of the Injection taken by the formula:

$$10(I / SV)$$

in which I is the absorbance of the solution from the Injection at the wavelength of maximum absorbance at about 268 nm; S is that of the solution from the *Standard preparation*; and V is the volume, in mL, of Injection taken. The content of total alkaloids is not more than 114.0% of the labeled amount of $C_{33}H_{40}N_2O_9$, and does not differ by more than 10.0% from the amount of $C_{33}H_{40}N_2O_9$ determined in the *Assay.*

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