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Reserpine Tablets

DEFINITION
Reserpine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$).

IDENTIFICATION

• **A.**

Sample solution: Evaporate about 2 mL of *Sample solution 2*, obtained from the test for *Other Alkaloids*, in a test tube to dryness. Add to the residue 0.5 mL of glacial acetic acid, and swirl for 1–2 min.

Analysis: Add 1 mL of a 20 mg/mL of vanillin in hydrochloric acid.

Acceptance criteria: A pink color is produced that turns to deep violet-red within a few min or as a result of warming the solution for 10–20 s.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and ammonium chloride solution (10 mg/mL) (1:1). The pH of the resulting solution is about 5.6.

Standard solution: 10 µg/mL of USP Reserpine RS in *Mobile phase*

Sample solution: 10 µg/mL of reserpine in *Mobile phase* from powdered Tablets (NLT 20). Pass through a suitable filter of 0.8-µm pore size.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) in the portion of Tablets taken:

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

r_U = peak response of reserpine from the *Sample solution*

r_S = peak response of reserpine from the *Standard solution*

C_S = concentration of USP Reserpine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of reserpine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution ⟨711⟩**

[Note—Do not substitute membrane filters for filter paper where the filtration of reserpine-containing solutions is indicated. Reserpine has been shown to be adsorbed onto membranes.]

Test 1

Medium: 0.1 N acetic acid; 500 mL

Apparatus 1: 100 rpm

Time: 45 min

***p*-Toluenesulfonic acid solution:** 10 mg/mL of *p*-toluenesulfonic acid in glacial acetic acid

Standard solution: 0.1 µg/mL of USP Reserpine RS in glacial acetic acid

Sample solution: Pipet an aliquot of the filtered solution under test, containing about 11 µg of reserpine, into a 125-mL separatory funnel. Extract with three 10-mL portions of chloroform, collecting the extracts in a 100-mL volumetric flask, dilute with glacial acetic acid to volume, and mix.

Analysis: Pipet 10 mL each of the *Standard solution*, the *Sample solution*, and glacial acetic acid into three individual 50-mL test tubes. To each tube, add 10 mL of *p-Toluenesulfonic acid solution*, insert a stopper, and mix gently. Place in a steam bath for 10 min. Remove from the steam bath, and cool. Determine the amount of reserpine ($C_{33}H_{40}N_2O_9$) dissolved from the fluorescences of the *Standard solution* and *Sample solution* using a suitable spectrophotometer arranged to deliver activation radiation at 390 nm and to measure the resultant fluorescence at the emission wavelength of about 480 nm.

Tolerances: NLT 75% (*Q*) of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus 1, *p*-Toluenesulfonic acid solution, Standard solution, Sample solution, and **Analysis:** Proceed as directed for *Test 1*.

Time: 30 min

Tolerances: NLT 80% *(Q)* of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$) is dissolved.

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

SPECIFIC TESTS

• **Other Alkaloids**

Adsorbent: Use acid-washed chromatographic siliceous earth.

Chromatographic tube: Chromatographic tube of 20 cm length and 22 mm internal diameter with an outlet at the bottom. Insert at the constriction a small pledget of glass wool previously washed with chloroform and air dried.

Chromatographic column: Mix 1 g of *Adsorbent* with 0.5 mL of freshly prepared sodium bicarbonate solution (20 mg/mL) in a 100-mL beaker until the mixture appears fluffy and uniformly moistened, transfer to the *Chromatographic tube*, and tamp lightly with a packing rod to a thickness of about 7–9 mm. Mix uniformly 1 g of *Adsorbent* with 0.5 mL of freshly prepared citric acid solution (5 mg/mL), transfer to the *Chromatographic tube*, and tamp lightly with a packing rod. Mix uniformly 1 g of *Adsorbent* with 0.5 mL of water, transfer to the *Chromatographic tube*, and tamp lightly with a packing rod.

Blank mixture: 1 mL of dimethyl sulfoxide and 2 g of *Adsorbent* in a suitable container. Stir until the mass is uniformly wetted and free from lumps.

Blank solution: Transfer the *Blank mixture* through a powder funnel to a prepared *Chromatographic column*. Scrub the beaker with about 1 g of *Adsorbent*, and add it through the funnel to the tube. Wipe the spatula, beaker, and funnel with a tuft of glass wool, previously washed with chloroform and air-dried. Place the glass wool in the tube, and press it down on the column with the packing rod, so that the overall height of the column is between 55 mm and 65 mm. Rinse the spatula, beaker, and funnel with the first portion of the chloroform used to elute the specimen. Elute the reserpine with 45 mL of chloroform. [Note—A properly packed column elutes in 4–8 min.] Collect the eluate in a 50-mL volumetric flask containing 14 mL of methanol. Rinse the tip of the column with chloroform, add chloroform to volume, and mix.

Standard stock solution: [Note—Use actinic glassware for this solution.] 0.1 mg/mL of USP Reserpine RS in methanol prepared as follows: Dissolve USP Reserpine RS in chloroform (0.01 mL/mg of USP Reserpine RS), and mix with previously warmed to 50° methanol (1.2 mL/mg of USP Reserpine RS). Transfer the mixture to a suitable volumetric flask with the aid of warm methanol, cool the solution to room temperature, dilute with methanol to volume, and mix.

Standard solution: [Note—Use actinic glassware for this solution.] 0.02 mg/mL of USP Reserpine RS from the *Standard stock solution* in chloroform and methanol. Initially add chloroform to about 72% of the volume of the flask, and then dilute with methanol to volume.

Sample solution 1: Weigh a portion of the finely powdered (powder should be able to pass through a 60-mesh sieve) Tablets (NLT 20), equivalent to about 1 mg of reserpine, but NMT 1 g of the powder, and transfer to a 150-mL beaker. Dry-mix the powder with about 500 mg of *Adsorbent*, then mix with 1 mL of dimethyl sulfoxide (immobile solvent). Stir thoroughly until the mass is uniformly wetted and free from lumps, and allow the mixture to stand for 5 min. Add another 500 mg of *Adsorbent*, and thoroughly work it into the mass. Again add an amount of *Adsorbent* so that the total amount added is 2 g, and disperse it completely in the mass.

Sample solution 2: Transfer *Sample solution 1* through a powder funnel to a prepared *Chromatographic column.* Proceed as directed for *Blank solution* beginning with “Scrub the beaker with about 1 g of *Adsorbent*.”

Analysis: Determine the UV absorption spectrum of *Sample solution 2*, between 255 and 350 nm, using the *Blank solution* in the reference cell. Similarly, determine the UV absorption spectrum of the *Standard solution*, using a solution of 3.6 volumes of chloroform and 1.4 volumes of methanol as the blank. The two spectra are similar, and the ratio, A_{268}/A_{295}, for *Sample solution 2* does not differ by more than 4.0% from the corresponding ratio for the *Standard solution*.

Calculate the percentage of reserpine ($C_{33}H_{40}N_2O_9$) in the portion of Tablets taken:

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

A_U = absorbance of *Sample solution 2* at the absorption maximum at about 268 nm

A_S = absorbance of the *Standard solution* at the absorption maximum at about 268 nm

Acceptance criteria: The result does not differ more than 6.0% from that obtained in the *Assay*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light resistant containers.
- **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP Reference Standards** ⟨11⟩
  USP Reserpine RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
RESERPINE TABLETS	Documentary Standards Support	SM22020 Small Molecules 2
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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