Diluent: Methanol and water (65:35)
Mobile phase: Methanol and *Buffer* (65:35)
Standard stock solution: 0.5 mg/mL of USP Altretamine RS in a mixture of methanol and water (70:30), prepared by first dissolving the Standard in methanol and then diluting with water to final volume.
Standard solution: 0.05 mg/mL of USP Altretamine RS in *Diluent*, from *Standard stock solution*
Sample stock solution: Transfer 25 mg of Altretamine to a 50-mL volumetric flask. Dissolve in 35 mL of methanol, and dilute with water to volume.
Sample solution: 0.05 mg/mL of Altretamine in *Diluent*, from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of altretamine ($C_9H_{18}N_6$) in the portion of Altretamine 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 USP Altretamine RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

Delete the following:

•• **Heavy Metals,** *Method II* ⟨231⟩**:** NMT 40 ppm •• (Official 1-Jan-2018)

SPECIFIC TESTS
• **Water Determination,** *Method I* ⟨921⟩**:** NMT 1%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Altretamine RS

Altretamine Capsules

DEFINITION
Altretamine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of altretamine ($C_9H_{18}N_6$).

IDENTIFICATION
• **A. Infrared Absorption** ⟨197K⟩
Sample: Remove as completely as possible the contents of 5 Capsules, and dissolve, with shaking, in 10 mL of chloroform. Filter, and evaporate the chloroform solution to dryness.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 0.79 g/L of ammonium carbonate in water. Adjust with a solution of formic acid (1 in 10) or ammonium hydroxide (1 in 10) to a pH of 8.0 ± 0.05.
Diluent: Methanol and water (65:35)
Mobile phase: Methanol and *Buffer* (65:35)
Standard stock solution: 0.5 mg/mL of USP Altretamine RS in a mixture of methanol and water (70:30), prepared by first dissolving the Standard in methanol and then diluting with water to final volume.
Standard solution: 0.05 mg/mL of USP Altretamine RS in *Diluent*, from *Standard stock solution*
Sample stock solution: Remove as completely as possible the contents of NLT 20 Capsules, and weigh. Mix the combined contents, and transfer as completely as possible to a 500-mL volumetric flask. Add 325 mL of methanol, and sonicate. Dilute with water to volume.
Sample solution: Transfer a volume of the *Sample stock solution*, equivalent to 10 mg of altretamine, to a 200-mL volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of altretamine ($C_9H_{18}N_6$) in the portion of Capsules 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 USP Altretamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Detector: UV 242 nm
Standard solution: USP Altretamine RS in *Medium*
Analysis: Determine the amount of $C_9H_{18}N_6$ dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted if necessary with *Medium*, compared with the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of $C_9H_{18}N_6$ is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.