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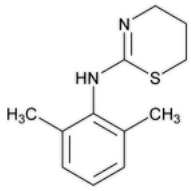
Xylazine



$C_{12}H_{16}N_2S$ 220.33

4*H*-1,3-Thiazin-2-amine, *N*-(2,6-dimethylphenyl)-5,6-dihydro-;

5,6-Dihydro-2-(2,6-xylidino)-4*H*-1,3-thiazine CAS RN®: 7361-61-7; UNII: 2KFG9TP5V8.

DEFINITION

Xylazine contains NLT 98.0% and NMT 102.0% of xylazine ($C_{12}H_{16}N_2S$).

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K**▲ (CN 1-May-2020)

Change to read:

• **B.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U**▲ (CN 1-May-2020)

Sample solution: 5 µg/mL in 0.1 N hydrochloric acid

Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**

Solution A: Dissolve 3.03 g of sodium 1-heptanesulfonate in 800 mL of water, adjust with 2 N sulfuric acid to a pH of 3.0, and dilute with water to 1000 mL. Pass through a filter of 0.5-µm or finer pore size.

Solution B: Acetonitrile

Diluent: *Solution A* and *Solution B* (50:50)

Mobile phase: See *Table 1*. Return to original conditions, and equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
10	60	40
15	60	40

Standard solution: 0.4 mg/mL of USP Xylazine RS in *Diluent*

Sample solution: 0.4 mg/mL of Xylazine in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 226 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of xylazine ($C_{12}H_{16}N_2S$) in the portion of Xylazine 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 Xylazine RS in the *Standard solution* (mg/mL)

C_U = concentration of Xylazine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

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

• **Limit of 3-Amino-1-propanol**

Standard solution: 0.5 mg/mL of 3-amino-1-propanol in methanol

Sample solution: 100 mg/mL of Xylazine in methanol. Sonicate to dissolve.

Chromatographic system

(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol and ammonium hydroxide (80:20)

Spray reagent: 2 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in a saturated chromatographic chamber containing the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and air-dry the plate. Spray the plate with the *Spray reagent*, and immediately heat the plate in an oven at 105°. When the spots are visible, remove the plate from the oven, and allow to cool.

Acceptance criteria: The intensity of the spot for 3-amino-1-propanol from the *Sample solution* is not greater than that of the spot for 3-amino-1-propanol from the *Standard solution* (0.5%).

• **Limit of Acetone and Isopropyl Alcohol**

Proceed as directed in Residual Solvents ⟨467⟩.

Acceptance criteria

Acetone: NMT 0.02%

Isopropyl alcohol: NMT 0.2%

• **Organic Impurities**

Solution A, Solution B, and **Diluent:** Prepare as directed in the *Assay*.

Mobile phase: See *Table 2*. Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
8	75	25
35	30	70
40	30	70

Standard solution: 0.008 mg/mL of USP Xylazine RS in *Diluent*, prepared by diluting the *Standard solution* from the *Assay*

Sample solution: Transfer 100 mg of Xylazine into a 10-mL volumetric flask, add 5.0 mL of *Solution B,* and swirl to dissolve. Add 4 mL of *Solution A,* and swirl. Dilute with *Solution A* to volume, and mix.

Chromatographic system

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

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; packing L7 (use a guard column)

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Xylazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of any individual impurity from the *Sample solution* that is not present in the chromatogram of the *Diluent*

r_S = peak area of xylazine from the *Standard solution*

C_S = concentration of USP Xylazine RS in the *Standard solution* (mg/mL)

C_U = concentration of Xylazine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2,6-Dimethylaniline	0.8	1.4	0.5
Xylazine	1.0	—	—
Specified unknown	1.3	2.8	0.5
2,6-Dimethylphenyl isothiocyanate	2.0	2.7	0.5
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	1.0

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

• **Labeling:** Where it is intended for veterinary use only, the label so states.

• **USP Reference Standards ⟨11⟩**

USP Xylazine RS

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

Topic/Question	Contact	Expert Committee
XYLAZINE	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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