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Tiletamine and Zolazepam for Injection

» Tiletamine and Zolazepam for Injection is a sterile dry mixture of Tiletamine Hydrochloride and Zolazepam Hydrochloride. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of tiletamine ($C_{12}H_{17}NOS$) and zolazepam ($C_{15}H_{15}FN_4O$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Tiletamine Hydrochloride RS
USP Zolazepam Hydrochloride RS

Identification—Constitute a container of Tiletamine and Zolazepam for Injection with a volume of water sufficient to yield a test solution containing the equivalent of about 10 mg of tiletamine and 10 mg of zolazepam per mL. Prepare two Standard solutions containing in each mL 10 mg of USP Tiletamine Hydrochloride RS and 10 mg of USP Zolazepam Hydrochloride RS, respectively. Separately apply 2 µL of the test solution and the Standard solutions to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Place the plate in a saturated chamber containing ethyl acetate as the solvent system and lined with filter paper. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the plate to air-dry, and examine under short-wavelength UV light: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solutions.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.07 USP Endotoxin Unit per mg of combined tiletamine and zolazepam equivalents.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩**:** between 2.0 and 3.5, when constituted as directed in the labeling.

Water Determination, *Method I* ⟨921⟩**:** not more than 20 mg in a container containing the equivalent of 250 mg of tiletamine and 250 mg of zolazepam.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩ and for *Uniformity of Dosage Units* ⟨905⟩.

Assay—

Internal standard solution—Prepare a solution of tetraphenylethylene in chloroform containing 10 mg per mL.

Standard preparation—Transfer accurately weighed quantities of about 116 mg of USP Tiletamine Hydrochloride RS and 113 mg of USP Zolazepam Hydrochloride RS to a 250-mL flask, add 2 mL of water, and swirl to dissolve. Add 30 mL of *Alkaline borate buffer*, *pH 10.0* (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and swirl. Add 5.0 mL of *Internal standard solution* and 95.0 mL of chloroform, and shake by mechanical means for 30 minutes. Allow the phases to separate, and use the chloroform layer as the *Standard preparation*.

Assay preparation—Constitute a container of Tiletamine and Zolazepam for Injection with the volume of water specified in the labeling. Transfer an accurately measured volume of the resultant solution, equivalent to about 100 mg of tiletamine and 100 mg of zolazepam, to a 250-mL flask. Add 30.0 mL of *Alkaline borate buffer*, *pH 10.0* (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and swirl. Add 5.0 mL of *Internal standard solution* and 95.0 mL of chloroform, and shake by mechanical means for 30 minutes. Allow the phases to separate, and use the chloroform layer as the *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.24-m column that contains 3% phase G2 on 100- to 120-mesh support S1AB. Helium is used as the carrier gas flowing at a rate of about 40 mL per minute. The column temperature is maintained at about 150° for 0.5 minute after injection and is programmed to rise to 230° at a rate of 10° per minute. The injector port is maintained at 160° and the detector at 250°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for tiletamine, 0.8 for zolazepam, and 1.0 for tetraphenylethylene.

Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of tiletamine ($C_{12}H_{17}NOS$) in each mL

of the constituted solution taken by the formula:

$$(223.33/259.79)(W/V)(R_U/R_S)$$

in which 223.33 and 259.79 are the molecular weights of tiletamine base and tiletamine hydrochloride, respectively; *W* is the weight, in mg, of USP Tiletamine Hydrochloride RS taken to prepare the *Standard preparation; V* is the volume, in mL, of the constituted solution taken to prepare the *Assay preparation;* and R_U and R_S are the peak area response ratios of the tiletamine peak to the tetraphenylethylene peak obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of zolazepam ($C_{15}H_{15}FN_4O$) in each mL of the constituted solution taken by the formula:

$$(286.31/322.77)(W/V)(R_U/R_S)$$

in which 286.31 and 322.77 are the molecular weights of zolazepam base and zolazepam hydrochloride, respectively; *W* is the weight, in mg, of USP Zolazepam Hydrochloride RS taken to prepare the *Standard preparation; V* is the volume, in mL, of the constituted solution taken to prepare the *Assay preparation;* and R_U and R_S are the peak area response ratios of the zolazepam peak to the tetraphenylethylene peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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

Topic/Question	Contact	Expert Committee
TILETAMINE AND ZOLAZEPAM FOR INJECTION	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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