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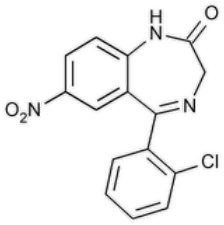
Clonazepam



$C_{15}H_{10}ClN_3O_3$ 315.71

2*H*-1,4-Benzodiazepin-2-one, 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-.

5-(*o*-Chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one CAS RN®: 1622-61-3; UNII: 5PE9FDE8GB.

» Clonazepam contains not less than 98.0 percent and not more than 102.0 percent of $C_{15}H_{10}ClN_3O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, at room temperature.

USP Reference standards ⟨11⟩—

USP Clonazepam RS

USP Clonazepam Related Compound A RS

3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyril.

$C_{15}H_{10}ClN_3O_3$ 315.72

USP Clonazepam Related Compound B RS

2-Amino-2'-chloro-5-nitrobenzophenone.

$C_{13}H_9ClN_2O_3$ 276.68

USP Clonazepam Related Compound C RS

2-Bromo-2'-(2-chlorobenzoyl)-4'-nitroacetanilide.

Change to read:

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

Melting range ⟨741⟩: between 237° and 240°.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

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

Limit of clonazepam related compound C—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Dissolve an accurately weighed quantity of Clonazepam in acetone to obtain a solution having a concentration of 25 mg per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Clonazepam Related Compound C RS in acetone to obtain a solution having a known concentration of 50 µg per mL.

Application volume: 20 µL.

Developing solvent system: a mixture of acetone and *n*-heptane (3:2).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. After air-drying the plate, heavily spray the plate with 2 M sulfuric acid, and dry at 105° for 15 minutes. Successively spray the plate with 0.01 M sodium nitrite, 9 mM ammonium sulfamate, and *N*-(1-naphthyl)ethylenediamine dihydrochloride TS, and dry the plate with a current of air. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of the *Standard solution:* no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from the *Standard solution* (0.2%).

Related compounds—

Buffer solution, *Mobile phase*, *Diluent*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Test preparation—Use the *Assay preparation*.

Procedure—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Clonazepam taken by the formula:

$$100Pr_i/(r_C + \Sigma Pr_i)$$

in which P is the relative response factor, which is 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; r_i is the peak response for each impurity obtained from the *Test preparation;* and r_C is the peak response for clonazepam in the *Test preparation:* not more than 0.1% of clonazepam related compound A or of clonazepam related compound B is found, not more than 0.2% of any other impurity is found, and the sum of all other impurities is not more than 0.3%.

Assay—

Buffer solution—Transfer about 6.6 g of anhydrous dibasic ammonium phosphate to a 1-L volumetric flask, dissolve in 950 mL of water, adjust with 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 8.0, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution*, methanol, and tetrahydrofuran (60:52:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of water, methanol, and tetrahydrofuran (60:52:13).

Standard preparation—Dissolve an accurately weighed quantity of USP Clonazepam RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL.

System suitability solution—Dissolve suitable quantities of USP Clonazepam Related Compound A RS, USP Clonazepam Related Compound B RS, and USP Clonazepam RS in *Diluent* to obtain a solution containing about 0.04 mg per mL of each Reference Standard.

Assay preparation—Transfer about 10 mg of Clonazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 2.2 for clonazepam related compound A, 2.5 for clonazepam related compound B, and 1.0 for clonazepam; and the resolution, *R,* between clonazepam related compound A and clonazepam related compound B is not less than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor 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 50 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 $C_{15}H_{10}ClN_3O_3$ in the portion of Clonazepam taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Clonazepam 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.

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

Topic/Question	Contact	Expert Committee
CLONAZEPAM	Documentary Standards Support	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

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