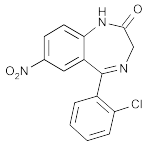
Clonazepam



$C_{15}H_{10}ClN_3O_3$ 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 [1622-61-3].

» Clonazepam contains not less than 98.0 percent and not more than 102.0 per cent 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.

Identification, *Infrared Absorption* ⟨197K⟩.

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%.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

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.

Clonazepam Oral Suspension

» Clonazepam Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$). Prepare Clonazepam Oral Suspension 0.1 mg per mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Clonazepam	10 mg
Vehicle: a mixture of Vehicle for Oral Solution, (regular or sugar-free), *NF* and Vehicle for Oral Suspension, *NF* (1:1), a sufficient quantity to make	100 mL

If using Tablets, comminute the Tablets into a fine powder in a suitable mortar, or add Clonazepam powder to the mortar. Add approximately 10 mL of the Vehicle, and mix to a uniform paste. Add the Vehicle in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough Vehicle to bring to final volume, and mix well.

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature, or in a cold place.

Labeling—Label it to state that it is to be well shaken before use, and to state the beyond-use date.

USP Reference standards ⟨11⟩**—**
USP Clonazepam RS

pH ⟨791⟩**:** between 3.6 and 4.6.

Beyond-use date: 60 days after the day on which it was compounded.

Assay—

Mobile phase—Prepare a filtered and degassed solution of water, methanol, and acetonitrile (4:3:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Dissolve an accurately weighed quantity of USP Clonazepam RS in acetonitrile to obtain a concentration of about 0.5 mg per mL.

Standard preparation—Dilute the *Standard stock preparation* with acetonitrile to obtain a solution having a known concentration of 25 µg per mL.

Assay preparation—Agitate the container of Oral Suspension for 30 minutes on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70 ° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 seconds. Pipet 2.5 mL of the sample solution into a 10-mL volumetric flask, and dilute with acetonitrile to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm analytical column that contains 5- µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the retention time of clonazepam is about 7 minutes; and the relative standard deviation for replicate injections is not more than 1.8%.

Procedure—Separately inject equal volumes (about 20 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 clonazepam ($C_{15}H_{10}ClN_3O_3$) in the volume of Oral Suspension taken by the formula:

$$4(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation; V* is the volume, in mL, of Oral Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Clonazepam Tablets

» Clonazepam Tablets contain not less than 90.0 percent and not more than 110.0 per cent of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$).

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

Identification—

A: Place an amount of finely powdered Tablets, equivalent to about 10 mg of clonazepam, in a 125-mL separator. Add 25 mL of water, shake for 2 minutes, and extract with two 40-mL portions of chloroform. Pass the extracts through anhydrous sodium sulfate, combine them, and evaporate at room temperature with the aid of a stream of nitrogen to dryness. Wash the residue with three 10-mL portions of solvent hexane: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Clonazepam RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩**—**

Medium: degassed water; 900 mL.

Apparatus 2: 75 rpm.

Time: 45 minutes.

Determine the amount of Clonazepam dissolved, using the following method.

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and acetonitrile (40:30:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Prepare a solution of USP Clonazepam RS in methanol having a known concentration of about 0.05 mg per mL. Quantitatively dilute a portion of this solution with *Dissolution Medium* to obtain a *Standard solution* having a known concentration similar to the expected concentration in the solution under test.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%; and the tailing factor is not more than 2.0.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the solution under test into the chromatograph, record the chromatograms, and measure the