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Apraclonidine Ophthalmic Solution

» Apraclonidine Ophthalmic Solution is a sterile, aqueous solution of Apraclonidine Hydrochloride. It contains an amount of apraclonidine hydrochloride ($C_9H_{10}Cl_2N_4 \cdot HCl$) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of apraclonidine ($C_9H_{10}Cl_2N_4$).

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

USP Reference standards ⟨11⟩—
USP Apraclonidine Hydrochloride RS

Identification—

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

B: Apply 2 µL of Apraclonidine Ophthalmic Solution and 2 µL of a Standard solution of USP Apraclonidine Hydrochloride RS in methanol containing about 11.5 mg per mL to a suitable high performance thin-layer chromatographic plate (see *Chromatography ⟨621⟩*) coated with a 0.2-mm layer of chromatographic silica gel mixture, or equivalent. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (74:22:4) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light. [Note—The apraclonidine spot should appear as a blue spot.] Spray the plate with fluorescamine solution, prepared by dissolving about 25 mg of fluorescamine in 25 mL of acetone. [Note—Avoid prolonged or repeated breathing of the aerosol from the fluorescamine spray. Also avoid prolonged or repeated contact with skin. Fluorescamine solution should be sprayed only in a hood.] Examine the plate under normal light and long-wavelength UV light. [Note—The apraclonidine spot should appear as a yellow spot under normal light and as a white spot under long-wavelength UV light.] The R_F value and appearance of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

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 4.4 and 7.8.

Assay—

Phosphate buffer—Prepare as directed in the test for *Chromatographic purity* under *Apraclonidine Hydrochloride*.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer*, acetonitrile, and methanol (68:30:2). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Standard preparation—Dissolve an accurately weighed quantity of USP Apraclonidine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a *Stock standard solution* having a known concentration of about 0.23 mg per mL. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 11.5 µg of USP Apraclonidine Hydrochloride RS per mL (equivalent to about 10 µg of apraclonidine per mL).

Resolution solution—Transfer about 1 mL of propiophenone to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of the *Stock standard solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of apraclonidine, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 254-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for apraclonidine and 1.0 for propiophenone; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 2.2; the resolution, *R,* between the analyte and propiophenone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

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 areas for the major peaks. Calculate the quantity, in mg, of apraclonidine ($C_9H_{10}Cl_2N_4$) in each mL of the Ophthalmic Solution taken by the formula:

$$(245.11/281.57)(2C/V)(r_U/r_S)$$

in which 245.11 and 281.57 are the molecular weights of apraclonidine and apraclonidine hydrochloride, respectively; *C* is the concentration, in µg per mL, of USP Apraclonidine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the apraclonidine 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
APRACLONIDINE OPHTHALMIC SOLUTION	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:
Pharmacopeial Forum: Volume No. Information currently unavailable

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