Guanfacine Hydrochloride

C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>O · HCl 282.55
Benzenacetamide, N-(aminooiminomethyl)-2,6-dichloro-,
N-(aminoiminomethyl)-2,6-dichlorophenylacetamide monohydrochlo-
ride [29110-48-3].

Guanfacine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>O · HCl, calculated on the dried basis.

Identification—
A: Infrared Absorption (197K).
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.

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%.

NOTE—Prepare all solutions in the tests for Related compounds and Chromatographic purity immediately prior to use, and apply to plates as quickly as possible.

Related compounds—
Spray reagent—[Caution—Avoid contact with α-tolidine. Prepare and use this Spray reagent in a well-ventilated hood.] Dissolve 50 mg of α-tolidine in 100 mL of alcohol, and mix.

Chlorine chamber—Transfer 1.5 g of potassium permanganate to a 100-mL beaker, dissolve in and dilute with water to volume, and mix. Transfer 25 mL of this solution to a beaker, and place the beaker inside a chromatographic chamber. Pipet 10 mL of hydrochloric acid into the beaker, and cover the chamber.

Developing solvent system—Prepare a fresh mixture of ethyl acetate, glacial acetic acid, and acetonitrile (70:25:3).

Standard solutions—Dissolve accurately weighed quantities of USP Guanfacine Hydrochloride RS and guanidine hydrochloride in methanol to obtain a solution having a known concentration of 0.4 mg each of USP Guanfacine Hydrochloride RS and guani-
dine hydrochloride per mL. Quantitatively dilute this solution with methanol to obtain Standard solutions having the following compositions:

<table>
<thead>
<tr>
<th>Standard Solution</th>
<th>Dilution</th>
<th>Concentration (µg RS and Guanidine Hydrochloride per mL)</th>
<th>Percentage (% for Comparison with Test Specimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(undiluted)</td>
<td>400</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>(1 in 2)</td>
<td>200</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>(1 in 4)</td>
<td>100</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>(1 in 8)</td>
<td>50</td>
<td>0.25</td>
</tr>
</tbody>
</table>

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

USP Reference standards (11)—
USP Guanfacine Hydrochloride RS

Test solution—Dissolve accurately weighed quantity of Guanfacine Hydrochloride in methanol to obtain a solution having a concentration of about 20 mg per mL.

Procedure—Use a thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.25-mm layer of chromatographic silica gel. Prewash the plates by placing in a chromatographic chamber saturated with Developing solvent system. Remove the plates from the chamber, and allow to dry y. Separately apply 10 µL each of the Standard solutions and the Test solution to the chromatographic plate. Allow the spots to dry y, and develop the chromatograms in Developing solvent 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, and allow the plate to air-dry y for about 1 hour. Examine the plate under short-wavelength UV light. Place the dried plate in the Chlorine chamber for 15 minutes, remove, and allow the excess chlorine to evaporate by air drying for 5 minutes. Spray the plate with Spray reagent, and examine: any spot due to guanidine hydrochloride observed in the chromatogram of the Test solution is not greater in size or intensity than the guanidine hydrochloride spot obtained from Standard solution 3 (0.5%); no other individual impurity spot observed in the chromatogram of the Test solution is greater in size or intensity than the guanidine hydrochloride spot obtained from Standard solution 4 (0.25%); and the sum of all impurities found, including guanidine hydrochloride, is not more than 1.0%.

Chromatographic purity—
Spray reagent 1—Prepare a mixture of tertiary butyl alcohol and water (9:1).

Spray reagent 2—Dissolve 5 g of 4,4′-tetrarmethylidiamino-
nodiphenylmethane in 20 mL of glacial acetic acid, add 10 mL of water, and mix (Solution 1). Dissolve 6 g of potassium iodide in 120 mL of water, and mix (Solution 2). Dissolve 0.3 g of ninhydrin in 10 mL of glacial acetic acid, dilute with water to 100 mL, and mix (Solution 3). Mix Solution 1 and Solution 2, and add 9 mL of Solution 3.

Developing solvent system—Prepare a fresh mixture of hexanes, diisopropyl ether, toluene, and glacial acetic acid (60:30:5:3).

Reference solutions—Dissolve an accurately weighed quantity of 2,6-dichlorophenylacetic acid in a mixture of methanol and water (9:1) to obtain a solution having a concentration of 1 mg per mL (Reference solution 1). Quantitatively dilute this solution with a mixture of methanol and water (9:1) to obtain Reference solution 2.

Reference solution 2 and Reference solution 3 having known concentrations of 0.5 and 0.25 mg per mL of 2,6-dichlorophenylacetic acid, respectively.

Test solution—Prepare a solution of Guanfacine Hydrochloride in a mixture of methanol and water (9:1), containing 100 mg per mL.

Procedure—Use a thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.25-mm layer of chromatographic silica gel. Prewash the plates by placing in a chromatographic chamber saturated with Developing solvent system. Remove the plates from the chamber, and allow to dry y. Separately apply 25 µL of each of the Reference solutions and the Test solution to the chromatographic plate. Allow the spots to dry, and develop the chromatograms in the Developing solvent system 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, and allow the plate to air-dry y for 30 minutes. Examine the plate under short-wavelength UV light. Spray the plate with Spray reagent 1, wait for 1 minute, and then spray with Spray reagent 2. Place the wet plate under short-wavelength UV light for 10 minutes, remove, and observe under white light: no spot observed in the chromatogram of the Test solution, other than that due to guanfacine hydrochloride, is greater in size or intensity than the principal spot obtained from Reference solution 2 (0.5%); and the sum of all impurities found is not more than 1.0%.
Assay—

Dilute phosphoric acid—Prepare a mixture of water and phosphoric acid (4:1).

Buffer solution—Dissolve 68 g of monobasic potassium phosphate in water, dilute with water to 1000 mL, and mix. Dilute 100 mL of this solution with water to 1000 mL, add 5 mL of triethylamine, mix, and adjust with Dilute phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of Buffer solution and acetonitrile (79:21). Make adjustments if necessary (see System Suitability under Chromatography (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Guanfacine Hydrochloride RS in a mixture of acetonitrile and water (3:1) to obtain a solution having a known concentration of about 1 mg of USP Guanfacine Hydrochloride RS per mL. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with Mobile phase to volume, and mix.

Assay preparation—Transfer an accurately weighed quantity of about 50 mg of Guanfacine Hydrochloride to a 50-mL volumetric flask, dilute in and with a mixture of acetonitrile and water (3:1) to volume, and mix. Transfer 2.0 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 220-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the responses as directed for Procedure: the capacity factor, k', is between 2 and 5; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2; 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 C₉H₉Cl₂N₃O · HCl in the portion taken by the formula:

\[ 1.25C(r_0/r_1)^2 \]

in which C is the concentration, in µg per mL, of USP Guanfacine Hydrochloride RS in the Standard preparation; and r₀ and r₁ are the guanfacine hydrochloride peaks obtained from the Assay preparation and the Standard preparation, respectively.

Guanfacine Tablets

Guanfacine Tablets contain an amount of Guanfacine Hydrochloride (C₉H₉Cl₂N₃O · HCl) equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of guanfacine (C₉H₉Cl₂N₃O).

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

USP Reference standards (11)—
USP Guanfacine Hydrochloride RS

Identification—

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

B: It responds to the Thin-Layer Chromatographic Identification Test (201), the test solution and the Standard solution being prepared at a concentration of 2 mg per mL in methanol, and a solvent system consisting of a mixture of ethyl acetate, glacial acetic acid, and water (5:2:2) being used.

Dissolution (711)—

Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Procedure—Determine the amount of C₉H₉Cl₂N₃O dissolved, employing the procedure set forth in the Assay and making any necessary modifications.

Tolerances—Not less than 75% (Q) of the labeled amount of C₉H₉Cl₂N₃O is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

pH 2.5 Diethyamine phosphate solution—Add 10.3 mL of diethyamine to about 70 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to 100 mL, and mix.

Reagent solution—Dissolve an accurately weighed quantity of 2,6-dichlorophenylacetic acid in Mobile phase, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 18 µg per mL.

Mobile phase—Dissolve 600 mg of monobasic potassium phosphate and 3 mL of pH 2.5 Diethyamine phosphate solution in 480 mL of water, and mix. Adjust with 0.2 N sodium hydroxide to a pH of 4.0. While swirling, add 520 mL of acetonitrile. Filter and degas. Make adjustments if necessary (see System Suitability under Chromatography (621)).

Internal standard solution—Prepare a solution of butylparaben in Mobile phase containing 0.5 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Guanfacine Hydrochloride RS in Mobile phase to obtain a solution having a known concentration of about 0.23 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, and add 5.0 mL each of the Reagent solution and the Internal standard solution. Dilute with Mobile phase to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of guanfacine, to a 100-mL volumetric flask. Add 50 mL of Mobile phase, and heat on a steam bath for 5 minutes. Cool to room temperature, dilute with Mobile phase to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative retention times are about 0.4 for guanfacine, 0.6 for 2,6-dichlorophenylacetic acid, and 1.0 for butylparaben; the resolution, Rₗ between guanfacine and 2,6-dichlorophenylacetic acid is not less than 1.5, and the resolution, Rₘ between 2,6-dichlorophenylacetic acid and butylparaben is not less than 1.5; 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 responses for the major peaks. Calculate the quantity, in mg, of guanfacine (C₉H₉Cl₂N₃O) in the portion of tablets taken by the formula:

\[ \frac{(246.09 + 282.55) + 0.25C(r_0/r_1)}{2} \]

in which 246.09 and 282.55 are the molecular weights of guanfacine and guanfacine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Guanfacine Hydrochloride RS in the Standard preparation; and r₀ and r₁ are the peak response ratios of guanfacine to butylparaben obtained from the Assay preparation and the Standard preparation, respectively.